M Y C E T O M A.

by

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C O N T E N T S .

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SECTION I.

HISTORICAL INTRODUCTION.

HISTORY.

Mycetoma has doubtless existed since the dawn of history and in fact has given rise to speculation as to the nature of pathological changes found in fossil remains. Brumpt (1943) quoting Blanchard's (1899) description of abnormal bones in a prehistoric horse 'Mergohippus Campestris' and Moodles (1922) account of similar changes in the fossilised skeleton of a rhinoceros embedded in pliocene rocks, went so far as to suggest that a form of mycetoma had been present. However, this suggestion does not bear close examination as the bone changes with which we are familiar in human cases at the present day are not actually pathognomic of the disease. A further important argument against Brumpt's hypothesis is that mycetoma is a disease unknown in veterinary literature.

Various authors have cited references to the condition in ancient Sanskrit texts such as the Alharvaveda, but those who have sought to verify these have been unable to do so. (Castellani 1919).

Because of the limited occurrence of the disease in temperate climates the omission of any mention of a condition like mycetoma from the Hippocratic texts is not surprising. This geographical excuse does not however apply to the writings of Avicenna and Rhazes as these two physicians frequented a part of the Arabian peninsula where the disease though by no means endemic is not unknown. Their references to Elephantiasis Arabium seem to apply to a filarial condition - this certainly was Galen's interpretation.

The absence of specific references, until comparatively modern times, to a disease which possesses a number of striking features such as the discharge of grains and characteristic deformities is nevertheless surprising. It is not until 1712 that Kaempfer gives a description of a condition which has

traditionally been accepted as the earliest record of mycetoma. In that year he published Amoenitatum Exoticorum describing journeys he had undertaken to the near and far East. When visiting Malabar, Ceylon, Japan and St. Thomas' Mount at Madras he was greatly struck by the number of natives with elephantine swellings of their lower limbs, some of which bore ulcers that discharged a serous fluid. He termed the condition Perical and concluded that it was endemic in regions where there were many volcanoes, hurricanes and earthquakes! In the absence of any mention of the grains which are such a characteristic feature of the condition, it seems probable that the sellings of the limbs which he noted could equally have been filarial as mycetomatous.

Even supposing that Kaempfer had been describing mycetoma his account was not in fact the first as a previous traveller John Fryer had some remarks to make about it in his "New Account of East India and Persia - being some years travels 1672 - 1681". (@) Kaempfer's Latin account when translated into English bears a strong resemblance to that of Fryer. Perhaps unjustly, I entertain a suspicion of plagiarism.

(@) D. McDonald in Trans. Roy. Soc. Trop. Med. & Hyg. 1955 49-(187-194), quoted extracts from Fryer's writings. It was after reading this article that I noted the similarity with Kaempfer's account.

Fryer saw at Madras " A cast of people living about St. Thomas Mount one of whose legs is as big as an elephant's which gives occasion for the divulging it to be a judgement on them as the generation of Assasins and Murtherers of the blessed apostle St. Thomas". However Fry does not seem to have been satisfied with this theological aetiology when he observed further cases along the Malabar coast. "Here also are those elephant legged St. Thomeans which the unbiased enquirers will tell you chance to them in two ways : By the venom of a certain snake, for which the Yogis or pilgrims, furnish them with a Factitious stone, which we call snake stone. As also by drinking bad water (to which as we do the Air, they attribute all diseases) ..... it is not much unlike the Elephantiasis Arabium".

Alard in 1809 published 'Histoire de L'Elephantiasis des Arabes', - a turgid monograph replete with dubious information as the author, never having left France, merely confused the very subject which he was investigating. One of the illustrations in the appendix is however perhaps of interest as it may be the first published of mycetoma. The author does not mention from what source he obtained the etching.

MADURA FOOT.

The first valid descriptions of mycetoma are to be found in the annual reports of the Madura Dispensary. Gill in 1842 described what was probably the actinomycotic form and gave details of the macroscopic internal changes. Colebrook, who had succeeded Gill in 1846, stated that the disease was known in Southern India as Madura Foot. This term is still in use in many parts of the world.

The early history of the disease in India was reviewed in detail by Carter in 1873 and by Castellani and Chalmers in 1919. A few of the most noteworthy points to be found in these accounts are mentioned below.

Garrison Surgeon Godfrey, of the Public Dispensary at Bellary, described in his annual departmental report

Reproduction of an illustration from the monograph of H.V.Carter (1874).

The infection in this case was caused by a black grain producing organism, presumably Madurella mycetomi. The original was drawn from life by Carter.

Fig 1.

of 1845 the presence of a black, coal-like deposit in a foot which he had amputated because of persistent ulcers presumed to be tuberculous.

In 1855 Ballingall, also in India, described a radiating form of granule present in discharges of an actinomycotic form of mycetoma. His writings became known on the continent and this led French authors to write of "La maladie de Ballingall" for mycetoma.

Rustomji, a military surgeon attached to Bhoo's Dispensary, near Kutch, first drew attention in 1858 to the two main types of the disease, one with black grains in the tissues and one with yellow grains.

VAN DYKE CARTER.

Carter has contributed more to our understanding of the disease than anyone else to date. His writings on the subject began in 1860 in the Transactions of the Bombay Medical Society and culminated in a fine monograph published in 1874. The latter work was beautifully illustrated by the author.

The foot of the first case that Carter studied is now in the Army Medical Museum at Millbank. Tissues were obtained from this specimen for histological study. Sections revealed grains which possess all the morphological characters of Madurella mycetomi. (Figure 37).

It was Carter who first recognised the fungal nature of the infection. Unfortunately he was led astray in his isolation of the responsible organism by growing a contaminant. He cultured a reddish mould, Chionyphe carteri (Berkeley), which is now unidentifiable. Carters error is not surprising in view of the ways in which he isolated this organism He was working at a time when laboratory techniques had not yet evolved as mycology was in its infancy and bacteriology was unknown. Even today, in spite of all the advances in laboratory methods and

Lateral view of one of Carter's cases. This specimen is now preserved in the Army Pathology Museum. The rods follow the track of sinuses. This same specimen is depicted in cross-section in the following illustration.

Fig.2

Sagittal section through the foot depicted on the preceding page. Several typical Madurella mycetomi cystic accumulation of grains are seen. A well defined capsule surrounds the loculus involving the calcaneus. Most of the grains have escaped from the focus beneath a cuneiform bone revealing a smooth and glistening inner wall. Though bone is affected there is no apparent destruction of articular cartilage.

Fig. 3

technology contamination of cultures is frequent and undoubtedly on occasions this is not recognised.

The clinical descriptions that Carter gives are excellent, and a number of pertinent features noted by him seem to have passed unobserved by later writers. His pathological descriptions were also very comprehensive. Carter noted that the 'black' form of the disease behaved differently, in some ways, from the 'yellow'. This particular point, which is developed later in the course of this paper, appears to have been ignored by all clinicians since Carter's time except for Grantham-Hill (1934).

KANTHACK.

Kanthack believed that the yellow and black forms of the disease were due to the same organism. He considered that the black grains were merely degenerate forms of the yellow granules. He only knew the disease from specimens sent to him at St. Bartholomew's Hospital, London and his lack of clinical experience may have contributed to his beliefs. His paper of 1893 was accompanied by excellent lithographs by the author and one of these suggested to me the appearance of a grain of Madurella grisea. I decided to study his material at first hand, and examination of his cases in the museum at St. Bartholomew's Hospital confirmed that amongst his limited material there had indeed been a case of Madurella grisea. This black grain is far closer in appearance to the yellow Streptomyces madurae than to the common black organism Madurella mycetomi. Because of this apparent resemblance he may well have thought that the grains of this particular case represented an intermediate form. Kanthack's case contains the first illustration of Madurella grisea. He also described grains of a micro-organism which he named Oospora indica which is probably synonomous with Streptomyces madurae. Mackinnon considers that in view of the priority of the name 'indica' over that of 'madurae' used by Vincent (1894) a case could

be made out for adopting the former designation. Unfortunately the term 'indica' has also been used for Streptomyces pelletieri and amendment would only lead to confusion. In any case Vincent did recognise that he was dealing with a new organism and was able to differentiate it from anaerobic actinomycosis.

Boyce and Surveyor in 1894 in a communication to the Royal Society showed that the 'white' actinomycotic form of the disease differed from the 'black' maduromycotic type. This fundamental morphological division has stood the test of time. The classification of the disease on the basis of grain colour was eventually realised to possess limitations, but the basic separation of the causative organism into Fungi imperfecta and those due to the Actimycetes is still valid.

SERIES OF CASES.

Only three series of over a hundred cases of this disease have been published.

Bocarro in 1893 reported his experience of mycetoma over a period of ten years at the Hyderabad Civil Hospital. This short article is merely a compilation of the site affected and possesses few clinical details.

Grantham Hill, (1934) a surgeon at Khartoum, described in the best paper that has yet appeared, the clinical and surgical aspects of mycetoma. His experience was however, limited to the three species occurring in that region.

Abbott (1953) at Wad Medani, also in the Sudan, has probably seen more examples of this disease than anyone else. His main interests have been in the mycological aspects of the disease and in trials of chemotherapeutic agents.

REVIEWS OF THE LITERATURE.

Comprehensive reviews of the literature have been equally scant. Carter (1874) and Musgrave and Clegg (1907) provide a bibliography of the cases occurring previous to the dates of their publications. No work embodying any full survey of papers on this subject has appeared since, largely owing to the difficulties in covering such widely dispersed articles in many languages.

There have been, however, a few papers which give a useful, albeit incomplete and selected review of this subject. Brumpt's Paris thesis of 1906 is excellent from the mycological aspects. Though many of his views are out of date he does describe a large number of species. Much of his discussion is based only on the morphology of the grains from single cases. It is an interesting exercise to try and classify some of these in the light of newer knowledge.

Yazbek.

Yazbek in a thesis of the University of Sao Paulo (1920) combines full clinical and mycological description of cases occurring in Brazil. His review of South American literature is particularly comprehensive.

Gammel, in 1927, reviewed much of the literature to that date, particularly that published in English. Though his personal experience of the disease was very limited his paper is helpful in summarising the number of organisms held responsible for the disease.

Since that date there have been numerous reports of further cases, the majority dealing with single instances whilst others have appeared devoted to the illumination of certain mycological aspects.

Mackinnon, a mycologist of Montevideo, has published many valuable papers in the last decade on

this subject. The basis of his work has been the comparison of numerous sub-cultures of different isolates from all over the world, in order to try and establish the attributes of each specie. Resulting from his studies many different species have been shown to be only artificial creations, as in fact they were identical with previously described organisms. This pioneer work has greatly reduced the synonymity which has so long obscured the field. Latterly he has become interested in the morphological appearances of the parasitic stages of these fungi.

So far there has been no attempt to correlate the clinical, pathological, radiological, and therapeutic aspects of mycetoma with the particular organism responsible. This has been largely due to the comparitive rarity of the disease in many areas, the limited geographical distribution of some of the species and the difficulties in surveying the literature. The present work is an attempt to assess the role of individual species in the various manifestations of the condition. It was for this reason that material and information was collected from many published and unpublished cases from all over the world.

SECTION II.

GENERAL MYCOLOGICAL INTRODUCTION.

MYCOLOGICAL ASPECTS OF MYCETOMA.

The following organisms have been found in mycetoma with sufficient frequency for them to be recognised as true aetiologic agents.

FUNGI IMPERFECTA.

Madurella mycetomi	Black or brown grains
Madurella grisea	Black or brown grains
Phialaphora jeanselmei	Black grains
Allescheria boydii (perfect form of Monosporium apiospermum)	White or yellow grains
Cephalosporium Sp.	White or yellow grains

ACTINOMYCETECEAE.

Nocardia asteroides	Yellowish grains
Nocardia brazilïensis	Yellowish grains
Streptomyces madurae	Yellowish grains
Streptomyces somaliensis	Yellowish grains
Streptomyces pelletieri	Pink or red grains.

There are numerous other species that have been incriminated but most have to be rejected from this list for reasons that will become evident in the following pages. There are a few organisms that occupy an uncertain position - notably the Aspergilli - and these will be discussed separately later.

These organisms are rarely discussed even in standard bacteriological texts so that before entering into details of the individual species a general discussion of certain broad features will not be out of place.

The systematic classification of the agents causing mycetoma is one of extreme complexity. A generally agreed classification on taxonomic grounds is far off and rightly so. If nomenclature were to be frozen on the basis of several irreconciliable current theories, the systematists would be deprived of the opportunity to revise their classification in the light of new knowledge.

As was shown by Boyce and Surveyor in 1894 the disease is due to two different groups of organisms, the molds and the actinomycetes. The maduromycetes possess coarse, clearly septate, branching filaments with well defined cell membrane and form chlamydospores whilst the actinomycetes have fine non-segmental filaments which are hyaline and show only a poor cell membrane.

FUNGI IMPERFECTA - Fuchel 1869

The fungi imperfecta are a heterogeneous group of molds distinguished from the Ascomycetes by the absence of a known sexual stage. Under current nomenclature fungi are classified mainly according to their perfect sexual form. Those Eumycetes in which this stage is unknown are placed in the Fungi imperfecta. With further knowledge it may be possible to greatly diminish the number of organisms in this group but without a perfect stage it is difficult to know upon what taxonomic features to place reliance. Some mycologists assume that these molds are merely the asexual stage of perfect fungi and group them according to alleged similarity with perfect forms. Monosporium apiospermum on culture sometimes develops ascospores and is then indistinguishable from Allescheria boydii. The identical nature of these two organisms is now agreed so that as the organism is called an Allescheria, strictly speaking it should not be termed a fungi imperfecta. This is however, an exceptional process occurring under certain, largely unknown circumstances. In some fungi imperfecta the ability to produce a

sexual stage may have been lost due to evolution.

NOMENCLATURE.

Species should be named according to the strict tenets of the International Rules of Botanical Nomenclature, but these have unfortunately often been disregarded in this field with resulting chaos owing to the number of synonyms created. The same specie is known under numerous eponyms which wrongfully designate merely separate strains rather than true differeing fungal entities. The extent to which this abuse has been practised can be judged from the eighteen different labels which are to be found in the literature attached to Allescheria boydii. Medical men tend to use the term Monosporium apiospermum for this specie as in human isolates it it usually ascocarpic. This clinical bias is of course anathema to botanical purists.

Further perplexities arise from the varying systems of taxonomy employed on the one hand by the British and American mycologists, and on the other by some Continental and Latin American workers. The latter following Vuillemin attach great importance upon alleged degrees of differentiation of the conidia and conidiophores whilst the former do not recognise these distinctions as valid.

In the past a great deal of the literature on fungi has been located in botanical journals and other sources not readily available to medical men. This still largely obtains as there is little liasion between these two main branches of mycology. Time may yet show that some of the maduromycoses are plant saprophytes or pathogens that have perhaps already been described. It is well known that on artificial media appearance may differ markedly from the mycelial parasitic stage. Perhaps some of the maduromycoses exist free in nature in another form.

PLEOMORPHISM.

Many isolates change greatly in morphology when maintained on culture media for a long time. This sometimes causes confusion when comparing subcultures of the same species preserved in culture collections as they may no longer appear similar as some have undergone pleomorphic changes. Madurella mycetomi, for example, is very unstable on culture. Following a variable interval some of the cultures become overgrown by a cotton-wool like mycelium which finally replaces the normal growth with the obliteration of many of the macroscopic and microscopic identifying structures such as conidia. Whether the strains are still pathogenic is not known as it is impossible to assess this factor. In the dermatophytes pathogenicity is not lost following pleomorphium, but even during animal passage the fungus does not revert to the original state.

A pleomorphic dermatophyte after growing on an 'earth' media can be converted to the form it possessed when originally isolated but if it is then transferred to Sabouraud slopes the pleomorphic form will reappear. (Salazar Leite).

The mechanism of pleomorphic transformation is obscure. Sugar containing media appear to encourage the process, whilst the so-called 'natural infusion media', which contain polysaccharides and complex carbohydrate moities, tend to inhibit the change. Sabouraud conservation agar is deficient in simple sugars for this reason.

Growth of the fungi imperfecti on earth and other low energy media would seem to merit further investigation. Recently Glenospora khartoumensis has been shown to be identical with Madurella mycetomi by such means (Abbott 1955, Mackinnon 1955)

PIGMENT PRODUCTION.

Much has been made of pigment production by fungal colonies as a means of identification. This was particularly emphasised in the past but now much less reliance is placed upon this feature.

Pigment production is subject to many variable factors many of which have not yet been fully investigated. Colonies of pleomorphic strains vary in the colour and degree of pigmentation. The colour varies with age and humidity of the cultures. Thus Cephalosporium falciforme on glucose agar first appears as yellow colonies, which over a period of a fortnight become buff, pink, violet, and finally brown. These particular changes, which occurred in subcultures I was growing, did not take place to the same degree on nutrient agar. It is thus obvious that the composition of the media is a factor of prime importance. For valid comparitive work a standardised preparation (such as Difco) is invaluable. The precise metabolites needed to form pigments in the mycetoma organisms have not been investigated to any extent. Certain amino-acids notably tyrosine, determine the degree of pigment formation in certain species. (Pipjer & Pullinger).

Because of so many variables the colour of the colonies is of limited value though it may provide a guide as to the organism. The production of an external diffusible pigment into the medium by some species, particularly Madurella mycetomi, is also of help in identification, but again it is not a constant feature.

In vivo the grains of each specie are generally of the same colour though rarely slight variations have been noted. Perhaps this constancy is due to a relatively fixed culture medium - the tissue fluids.

FERMENTATION REACTIONS.

The biochemical activity of most of the organisms have been studied but the ultimate value in diagnosis of a specie is difficult to assess. Such tests may eventually help to supplement the morphologic feature in establishing the identity of a fungus. Within a specie there is a range of variation in biochemical behaviour. There is a danger that any gain in precision resulting from such studies may be offset by the differentiation of further minor variants. Following the development of pleomorphic changes the fermentation reactions do not apparently alter (Mackinnon 1954).

Biochemical test should be performed under standardised conditions if comparison with the works of others is to be any use. False results, for example, can be avoided by reporting as positive only instances when gas is formed, as acidification alone may be due to other biochemical transformations besides fermentation of the sugar being investigated.

Proteolytic and amylolitic activity is more readily tested but even in such investigations there are conflicting reports. When Mackinnon publishes further data concerning his comparison of strains of each specie it may be possible to determine the range of variation within a specie.

The nutritional requirements are not accurately known for any of the fungi causing mycetoma.

CONTAMINANTS.

Ever since the day of Carter contaminants have been the source of numerous errors in the study of mycetoma.

Bacterial contamination is a nuisance as it may hinder or prevent the growth of the responsible fungus on a culture medium. The pH of the medium can be adjusted to deter the growth of bacteria but such a medium is probably not the best to use for primary

culture, though it is valuable in the conservation of subcultures.

If grains are procured aseptically from the centre of a lesion they are usually bacteriologically sterile in Madurella mycetomi infections. This procedure is naturally of limited application as the usual culture material is obtained from discharging sinuses. In such a case the grains should be rinsed in isotonic saline several times to remove gross surface contamination. This may be followed by a final rinse in a penicillin solution before placing the grain in a culture slope. Penicillin does not affect the growth of a fungi imperfecta to any degree (it has slight effect on some of the actinomycetes) but it will suppress many pyogens. An improvement on the above procedure is the introduction of antibiotics into the culture media. One such selective medium for the isolation of pathogenic fungi contains 25 units of Streptomycin and 6 units of penicillin per ml. (Boeing & Laffer 1947).

Fungal contaminants are far more troublesome. They may not be recognised as such, with the result that saprophytes or chance commensals have pathogenic potentialities attributed to them. When an isolate is found to belong to an omnipresent genus, such as the Cephalosporium, it should be viewed with scepticism unless certain criteria are fulfilled. The isolation should be repeated on several occasions if this is possible. The appearance of tissue grains should be compatible with the diagnosis. In a case of mycetoma in the U.S.A. an Aspergillus was isolated repeatedly from a case but the grains clearly indicated an actinomycete. The culture of Aspergillus niger was considered to be a fortuitous contaminant. (Reilly & Steel 1949).
There are other such cases in the literature.

In the literature there are frequent single reports of the isolation of a specie. Some of these were

probably the responsible organism but it is only right that pathogenicity should be considered sub-judice until the fungus is again noted.

Since the disease is rarely transmissible it is not possible to apply Koch's postulates as a test of pathogenicity.

ANIMAL INOCULATION.

The organisms appear to be poorly adapted for parisitic existence, judging from the rarity of the disease in humans and the virtual absence of any cases in the veterinary literature*. The difficulties encountered in reproducing the disease in animals is therefore no cause for surprise. Many methods of experimental infection have been employed but few have borne any similarity to the natural means of infection. Intracardiac or intravenous injection of mycelial extracts may be fatal of themselves by embolisation.

A foreign body reaction alone is insufficient evidence of infection, unless it can be shown that this reaction does not also occur with dead (autoclaved) fungal elements.

In the ideal method of reproducing the disease, scarification or subcutaneous inoculation of either spores or mycelial extract would lead to a chronic progressive granuloma producing young grains from which the original culture could be recovered. Intraperitoneal passage can also be used experimentally, but with some reservations. There should be

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* A tumour in a captive viperine snake was attributed to a specie of Cephalosporium. It proved possible to transmit the culture to another viper which subsequently died. Rodhain and Mattlet. Ann. Parasit. Hum. et Comp. 1950 - 25 - (77-79).

a reasonable interval before attempting to recover the strain from the animal to ensure that part of the original inoculum is not merely being passively transferred. It is known that spores can survive in animals for days, though mycelium dies more rapidly (Coutelen, Mackinnon 1954). Many experimental infections prove to be self limited. In some the tumour is due largely to persistence of the inoculum in association with a marked proliferative fibrous reaction.

The choice of experimental animals is probably important. Most work has been carried out on rabbits, mice and guinea-pigs, though a few workers have inoculated monkeys and even pigeons. (Musgrave and Clegg - Pinoy.)

There is a further technique of causing experimental infection which does not appear to have been employed with mycetoma organisms. Friedman et al., have shown that cortisone and X-rays enhance the virulence of certain test organisms with which they were working. Candida albicans for example could be rendered pathogenic by peritoneal inoculation if the animal had been irradiated and given cortisone beforehand, whereas without these preliminary measures nothing occurred. Blastomycosis, amongst the fungi tested, also showed a marked increase in virulence. The action of cortisone and radiation were found to be synergistic but the exact machanism was obscure. Alterations in the nature of the cellular response and of antibody production were suggested as explanations for the phenomenon by the authors.

From the foregoing it is obvious that knowledge of experimental infections with mycetoma organism is fragmentary. Further details regarding experimental infections are to be found in the descriptions of individual species. With two of the fungi imperfecti, Allescheria boydii and Phialaphora jeanselmei, experimental infections can probably be caused with some certainty (Gammel &

Moritz 1933; Gellman & Gammel 1933; Nino 1941; Ajello 1952 - 1954) The other species are much more uncertain in their effect. Madurella mycetomi has only caused lesions on three occasions and in none of these is the method above reproach. (Nicolle & Penoy 1908; Blanc & Brun 1919; Yazbek 1920.) Many other workers have attempted to infect animals with Madurella mycetomi and all have failed.

The results of infection in an experimental animal must be related to human cases with reservation. However, if a reliable way of causing experimental infections could be developed it would provide a means of in-vivo testing of the efficacy of drugs.

ACTINOMYCETES.

I will not discuss the problems presented by this group of organisms in such detail as those of the fungi imperfecti, as much of what has been written applies to both.

NOMENCLATURE.

Waksman(1950) lists 23 different generic names for these organisms and 16 differing schemes of classification. A simple calculation reveals the nightmarish number of possible synonyms presented by such a situation.

The systematic position of the Actinomycetes is still a matter for dispute. Some authorities claim them as bacteria, others as fungi and yet others believe them to be a transitional form between these two. The size of the filaments, which may show fragmentation and acid fastness, is reminiscent of the bacteria, whilst aerial mycelium with conidia formation is more suggestive of the fungi.

Gordon and her co-workers are, in Waksman's laboratory, attempting to delineate taxonomically the species represented by the Nocardia, Streptomyces, and the rapidly growing Mycobacterium so that they may be recognised regardless of their history, age or state of variation.

Groups of stable correlating characters have proved difficult to find. (Gordon 1955). These researches when concluded should prove interesting.

Rather than try to discuss these complex and controversial matters, which are of little clinical relevance, I will simply adopt the classification of Waksman and Henrici (1943).

A. MYCELIUM RUDIMENTARY OR ABSENT.	Mycobacteriaceae
I. Acid Fast Organisms	Mycobacterium
B. TRUE MYCELIUM PRODUCED.	
I. Vegetative mycelium fragmenting into bacillary or coccoid elements.	Actinomycetaceae (Buchanan).
a). Anaerobic, parasitic, not acid fast.	Actinomyces (Harz)
b). Aerobic, partially acid fast or non acid fast.	Nocardia (Trevisan)
II. Vegetative mycelium not fragmenting into bacillary or coccoid forms	Streptomycetaceae (Waksman & Henrici)
a). Multiplication by Conidia in chains from aerial hyphae	Streptomyces (W. & H.)
b) Multiplication by single terminal spores on short sporophores.	Micromonospora (Orskov)

The above is an all encompassing classification which includes the majority of the micro-organisms which are present in the soil. Very few of these are pathogenic. The following species are commonly recognised as causing mycetoma.

Aerobic semi acid fast.

Nocardia asteroides)
Nocardia braziliensis) yellow or white grains

Aerobic non acid fast.

Streptomyces madurae	}	yellow or
Streptomyces somaliensis	}	white grains
Streptomyces pelletieri	—	pink or red grains

All the organisms causing mycetoma are aerobes.

BLACK GRAIN ACTINOMYCOSIS.

There have been three black grain mycetomas reported which have not been caused by a true fungus.

Almeida and Torres treated such a case in which the infection had involved a shoulder and part of the chest wall. (Lacaz 1945). Illustrations reveal that the black grains are clearly due to an actinomycete as only filaments are seen and there are not septate hyphae. The organism was grown and termed Actinomycosis Paraguayensis (Almeida 1939). Gonzalez-Ochoa and Sandoval (1951) and later Mackinnon (1955) studied subcultures of the original strain and thought that a Streptomyces, resembling if not identical with Streptomyces albus, had been grown.

A further case was reported by Conti and Negroni (1951) in Brazil but I have been unable to procure this communiciation. A third case is also alleged to have occurred in Bucharest and was described by Mironescu but, again the original paper has been unavailable.

It would seem to be established that a specie of actinomycete can cause a black grain form of mycetoma but the nature of the responsible agent is still uncertain. Almeida's actinomycete may be a contaminant or may indeed represent the causal strain.

ACTINOMYCOSIS AND NOCARDIOSIS.

Classical Actinomycosis caused by the anaerobic, or facultative anaerobes, Actinomyces bovis and Actinomyces hominis are not considered to be a cause of mycetoma, though, pathologically and clinically, they show some analogies. The difference is that the

anaerobic infection is acquired endogenously and, as a rule, is retricted to definite sites. I have been unable to find any descriptions of peripheral limb involvement. The leading authority on Actinomycosis has never seen a case on the foot. (Cope 1955).

Even though the anaerobic organism does not cause mycetoma, the Nocardia can cause lesions somewhat akin to Actinomycosis of the cervico-facial, thoracic, cerebral and abdominal types. There may be only a single site involved or multiple areas may be affected suggesting a haematogenous dissemination. The latter form of the disease is termed Nocardiosis and probably represents a Nocardial septicaemia. It is surprising how rarely the localised mycetomatous lesions themselves give rise to the diffuse systemic infection. I have only been able to discover one such case (Bobbitt et al. 1955). It has been suggested that the thoracic Nocardial infections are acquired by spread of an external granuloma across the chest wall, though this is certainly so in a few cases the fundamental aetiology remains obscure. Nocardiosis is a progressive cachexial condition which proves fatal. Chemotherapy has improved the prognosis but the mortality is still very considerable.

NOCARDIA.

The genus was named after the French bacteriologist Nocard who first described the type specie Nocardia farcinucus. This aerobic, acid fast organism is responsible for the disease of cattle termed 'farcin de boeuf'.

On culture these organism reveal slender filaments or rods which are less than 1 μ in diameter. Branching rarely occurs, short rod forms being more characteristic, particularly in old cultures. Scarce coccal bodies represent spores which are formed by fission. Filaments stain readily and are strongly gram positive. With certain stains the coccal and rod forms can be

coloured differentially. Acid fastness is invariably present but varies greatly in degree. Nocardia asteroides will withstand more decolourisation than Nocardia brasiliensis.

STREPTOMYCES.

The name of this genus has become widely known with the discovery by Waksman of the antibiotic Streptomycin. Since then further antibiotics have been discovered in other species beside Streptomyces griseus.

Far more branching is seen in the vegetative mycelium of this genus. Hyphae are larger than those of Nocardia. Special techniques may reveal segmentation but this is thought to be merely a prelude to fragmentation. The most characteristic feature is the presence of an aerial mycelium which carries spores. The filaments do not stain as readily with Gram as those of Nocardia and no trace of acid fastness can be detected.

SECTION IIA.

RARE AND DEBATEABLE CAUSES OF MYCETOMA.

RARE AND DEBATABLE CAUSES OF MYCETOMA.

A number of individual case reports of mycetoma due to species other than those mentioned in the preceding section indicate that further pathogenic strains ought probably to be distinguished. Some reports can be discounted as the isolate subsequently proved to be a contaminant. Thus, Aspergillus chevallieri (Mangin 1909), was held to be the organism responsible for an Argentinian mycetoma studied by Negroni and Tey (1938), but a later study of the same patient showed that the condition had been caused by a well recognised pathogen (Negroni & Fernandez 1947). A black grain mycetoma occurring in Italy was attributed to Penicillium mycetogenum but it is likely that this was a contaminant (Mantelli and Negri, 1915). There are a number of other instances of this sort.

At other times a contaminant has probably not been responsible, as neither the fungus isolated nor the grains fit into any of the recognised pathogenic species. Mention has already been made of black grain actinomycosis and the conclusion then reached was that there exists a further pathogenic variety of Actinomycete, probably of the genus Streptomyces. A maduromycete producing black grains has been isolated on two occasions and the cultural features and the grain morphology are both at variance with Madurella sp. and Phialaphora jeanselmii. The first of these was reported by Gelonesi in 1927 from Italian Somaliland, and the second from Bucharest by Nicolan and Evolceanu in 1947.

Hemispora stellata was originally observed as a contaminant on a colony of Aspergillus repens by Vuillemin. The features by which it can be identified are well established and are summarised in a paper of Cifferi and Redaelli (1948). The preferred designation of the specie now appears to be Sporendonema sebi, but there are numerous synonyms.

The pathogenic status of this organism is in doubt, but it has been alleged to be the cause of granulomatous processes similar to sporotrichosis on several occasions (Fonseca and Area-Leao, 1928). In 1930 Sartory et al. recovered the specie from a mycetoma-like lesion of the ankle affecting bone.

Rubromadurella langeroni is a maduromycete which was found by Talice in Montevideo in a biopsy of a small nodule which had been present on a finger. Unfortunately it was not possible to carry out cultural work but, because of the orange red colour of the grains due to pigmented hyphal walls and chlamydospores he considered that this was a new specie. Mackinnon considers that in fact the grains were of a Monosporium stained by blood pigments. This is not an entirely satisfactory explanation as it is unlikely that haemoglobin derivatives could have penetrated throughout the interior of a grain. I feel that perhaps this may have been a Madurella grisea infection as some of the small grains of this specie show orange hyphae on section.

ASPERGILLOSIS.

Members of the genus Aspergillus have been reported from many types of human lesions and some species are established pathogens causing otomycosis, various forms of pulmonary suppuration, endocarditis, cerebral abscesses, meningitis and septicaemia. On several occasions Aspergilli have been implicated in a disease process which in some ways resembles mycetoma as black grains are present and a tumefaction is produced due to a chronic granuloma which may invade bone. But on each occasion metastatic lesions have developed a feature which alone would serve to distinguish it from the maduromycosis. Thus, Puestow (1929) describes a case in a student where lesions spread from the neck to the face and arms; Cartia (1930) mentions spread from one foot to the other and to a

forearm; Spediacci (1951) notes spread from a metatarsal to ribs and perhaps viscera. Basset et al. (1948) describe 'Mycetoma' due to an Aspergillus affecting three brothers. These patients were observed in Paris and will be described in detail as they exemplify the great virulence of the organism responsible. The first patient was infected at the age of eleven following a cut incurred whilst bathing. This did not heal, fistulae developed and the ankle joint was destroyed. The process then extended to the knee and eventually necessitated a mid-thigh amputation. Six years later the patient abraded the remaining ankle with his prosthesis and this wound in turn became similarly infected. Meanwhile the second brother, had contracted an identical infection at the site of an abrasion and lter he also required amputation of one leg and eventual surgical treatment on his other leg. At about the same period, following a thorn injury a third brother developed destructive lesions of one foot. The lamentable story may indeed have been as stated but doubts arise when one reads of the therapeutics applied and which apparently arrested the infection. After penicillin had proved unavailing recourse was made empirically to the following rather unorthodox polytherapy - a vaccine from B. subtilis, a substance derived from a Sarcinia and the root of Plumbago europa!

I have omitted from mention the specie described on the basis of grain morphology as Aspergillus bouffardi. This has been reported twice but on neither occasion was any culture obtained. (Brumpt 1906 and Balfour 1911). The grains depicted in these two papers reveal all the characteristic attributes of Madurella grisea. The original label of Aspergillus was applied as it was thought that typical 'aspergillar heads' were visible at the periphery of the grain. Characteristics of the cultural phases have not been noted in the parasitic stages of the other maduromycetes, so it seems unlikely the Aspergilli should be exceptional in this regard.

SECTION III.

MYCETOMA AND GEOGRAPHICAL FACTORS.

MYCETOMA AND GEOGRAPHICAL FACTORS.

An enquiry into the distribution of a disease so widespread as mycetoma encounters peculiar difficulties which tend to limit the accuracy and completeness of the information obtained. It so happens that the disease is sporadic and rare in highly developed communities, but it is just in these places that individual cases can be expected to be fully investigated and reported. Accounts in the literature reflect rather on the distribution of medical mycologists than the true incidence of the disease. Thus there have been over fifty cases in the last decade reported from the U.S.A., Mexico, and Brazil whilst only one has been noted as occurring in South West Arabia. This gives an entirely false picture of the incidence as the latter region is in fact productive of about 90 cases annually.

The absence of reported cases does not necessarily mean that the condition is in fact unknown in a given region. Standard works and papers frequently refer to the freedom of Oceania from mycetoma. This is largely true though Nocardia asteroides has infrequently been reported from Hawaii, the Philippines and Indonesia but the majority of the cases were not autochthonous. (Romulo, Hausman, Musgrave & Clegg, King). Because of the climate and the conditions pertaining to the local population, I felt that mycetoma might well occur amongst the Aboriginals of the Australian out-back, despite the absence of any published reports. Enquiry has shown that in fact Nocardial sp. mycetoma does in fact occur-indeed at Alice Springs alone, at least four cases have been seen in the last few years. (Welton)

The above remarks and examples illustrate that a case is most likely to be reported if it occurs where its rarity renders it, per se, remarkable.

Where the disease is common it is not primarily familiarity that has led to the lack of detailed investigations. Frequently adequate facilities are not available. More often there is little urge to describe a disease in which there is no specific therapy apart from a surgical excision which does not pose any problems.

From Africa particularly, there have been few reports which give details of species involved. This applies particularly to the British territories where medical mycological laboratories are non existent. Some day these may be established and then it will be possible to determine the exact species of Nocardia that occur there. The Belgian Government recently sponsored a mycological research expedition to the Congo (Vanbreuseghem). Many cases of fungal disease came to light which had never previously been reported from this part of Africa. Amongst these were mycetoma due to Allescheria boydii and a streptomyces.

To supplement the inadequate published data it has therefore been necessary to enquire widely of clinicians and pathologists in the tropics and subtropics regarding their own personal experience. In general the response has been excellent and many have been most generous in their co-operation, forwarding clinical details, histological materials and photographs. Nevertheless few replies have been received from certain territories such as the Levant, India and Pakistan and the Portugese colonies.

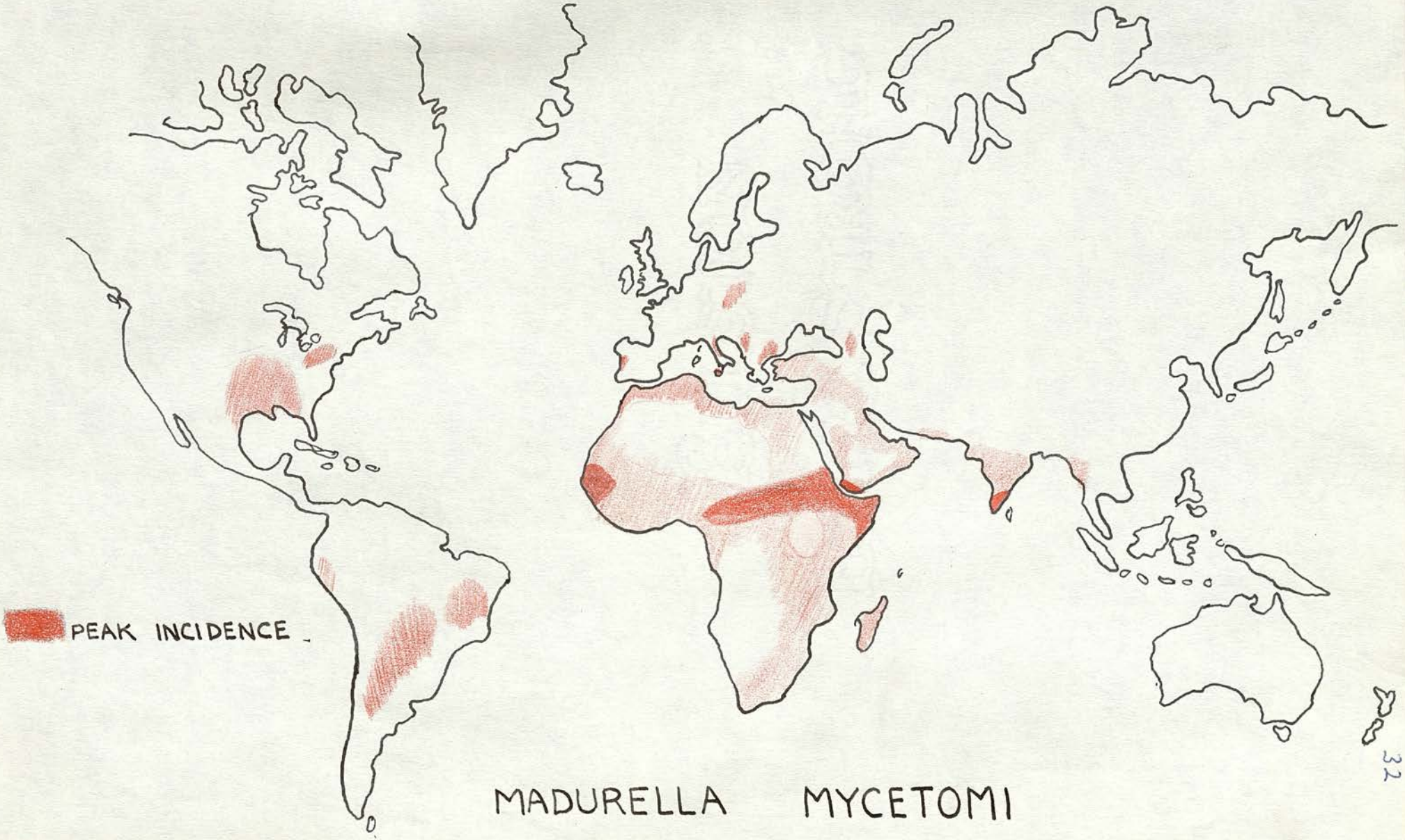
Taking all sources into consideration it seems as though at least 600 new cases occur in the world annually, the large majority being in the Sudan, Arabia and Southern India. Though this figure is undoubtedly an underestimate it does show that the disease is not so very uncommon. The most common organisms are Madurella mycetomi, Streptomyces madurae, and Streptomyces somaliensis.

The various species causing the disease show different geographical distribution. Some are apparently world wide as Nocardia asteroides, whilst others, like Streptomyces somaliensis, only present in a circumscribed area.

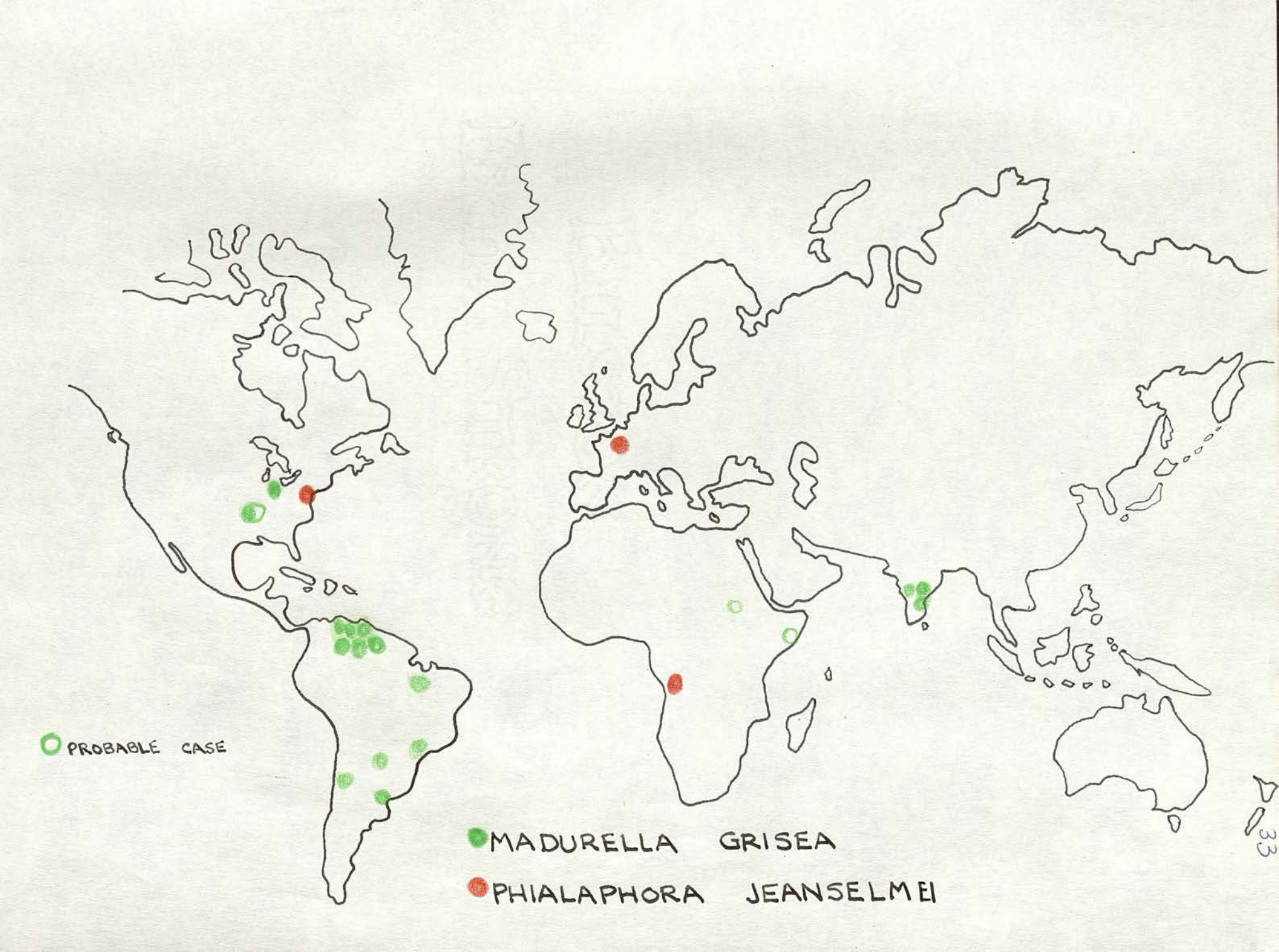
I have sought to show the localisation of some of the species in the accompanying maps. The Nocardia and Streptomyces madurae have been omitted as insufficient information is available to give an accurate picture. As previously stated the Nocardia seen in East and West Africa have not been adequately studied. Mycologically the tissue grains are suggestive of Nocardia braziliensis which to date has not been reported outwith the New World.

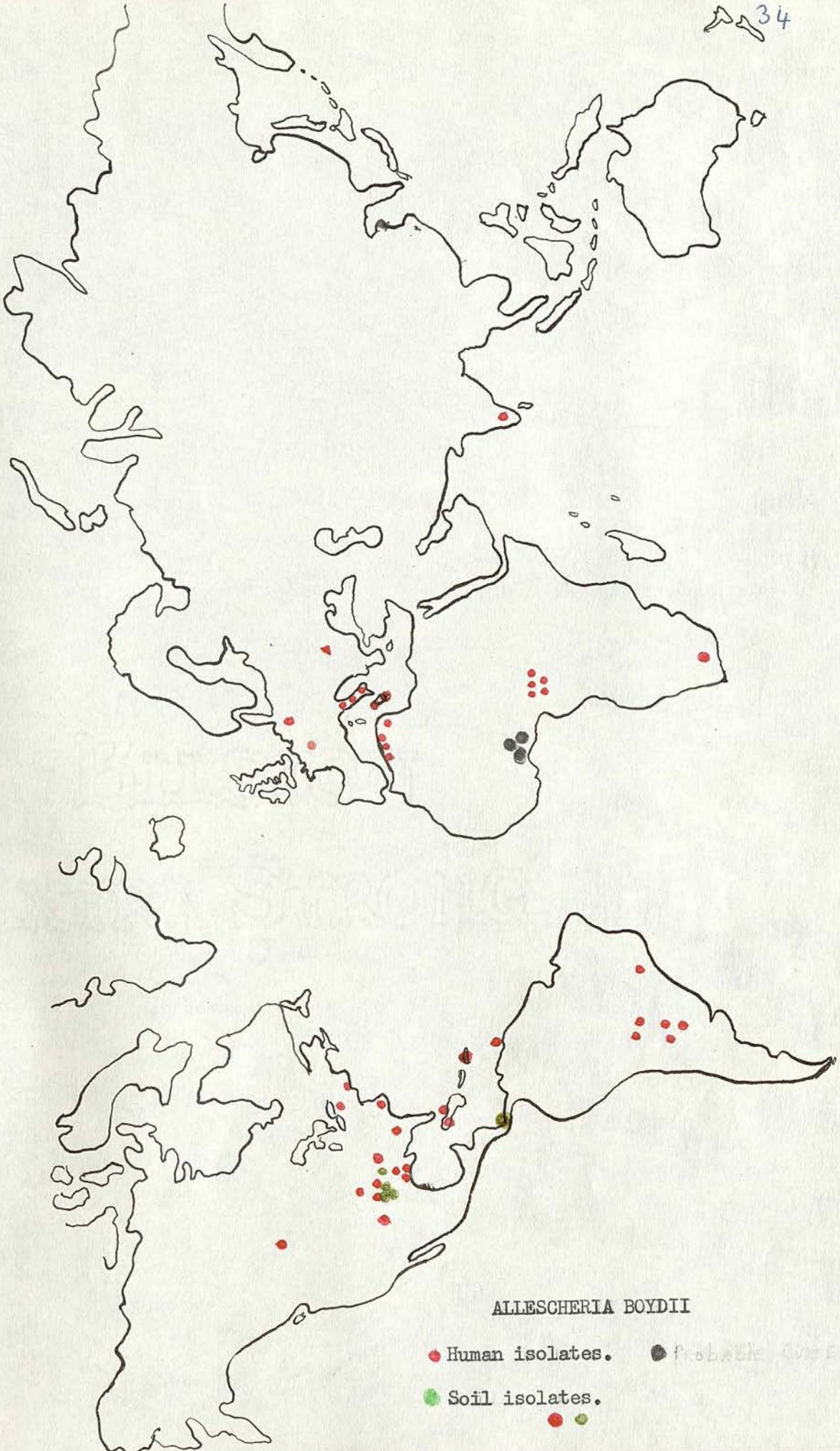
Fuller details regarding the distribution of each specie will be found later in the sections relating to the individual species.

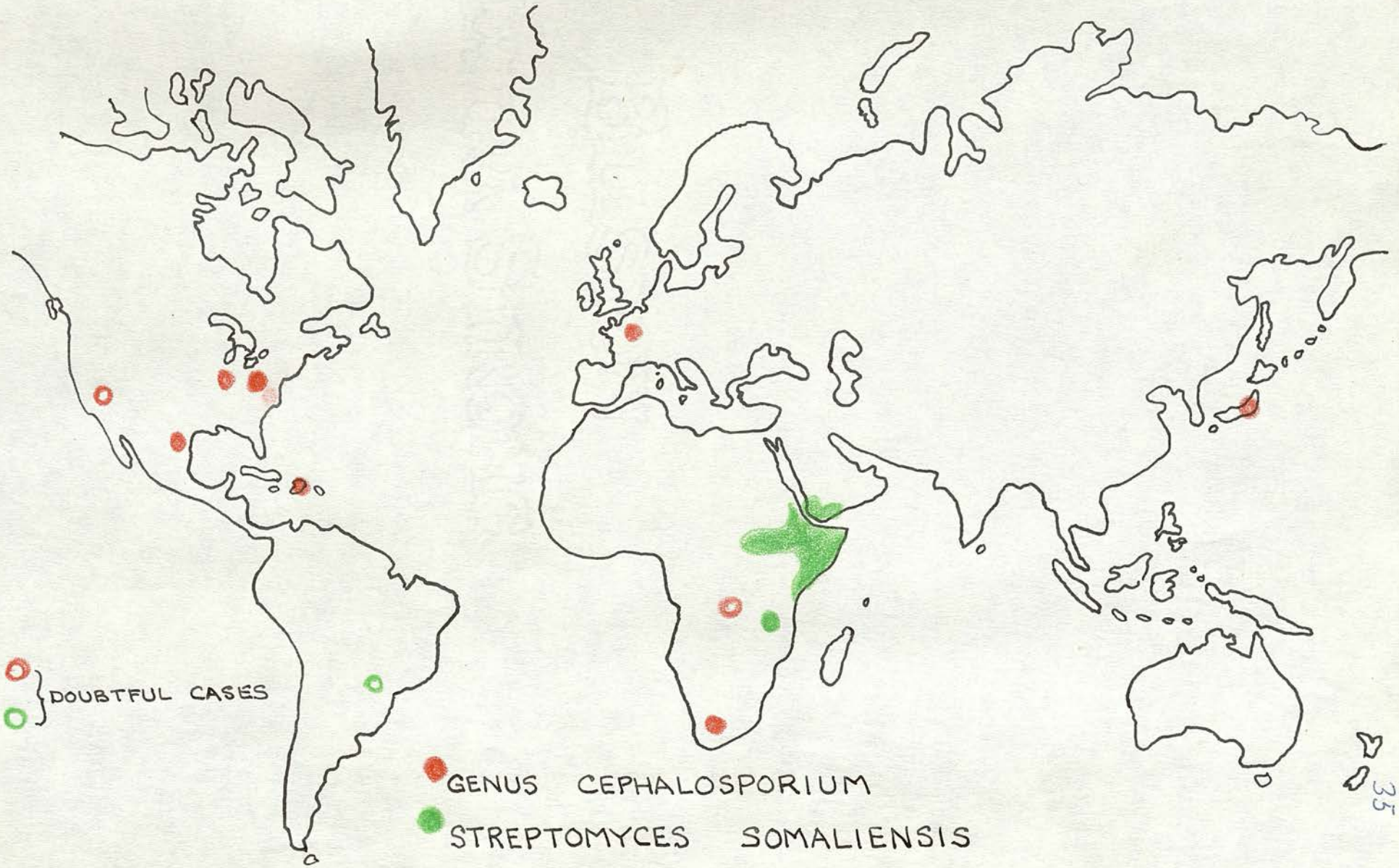
The map pertaining to Madurella mycetomi, Streptomyces pelletieri show the region where the disease occurs rather than individual cases. Parts where mycetoma is common have been differentiated from areas where only a single example has occurred.

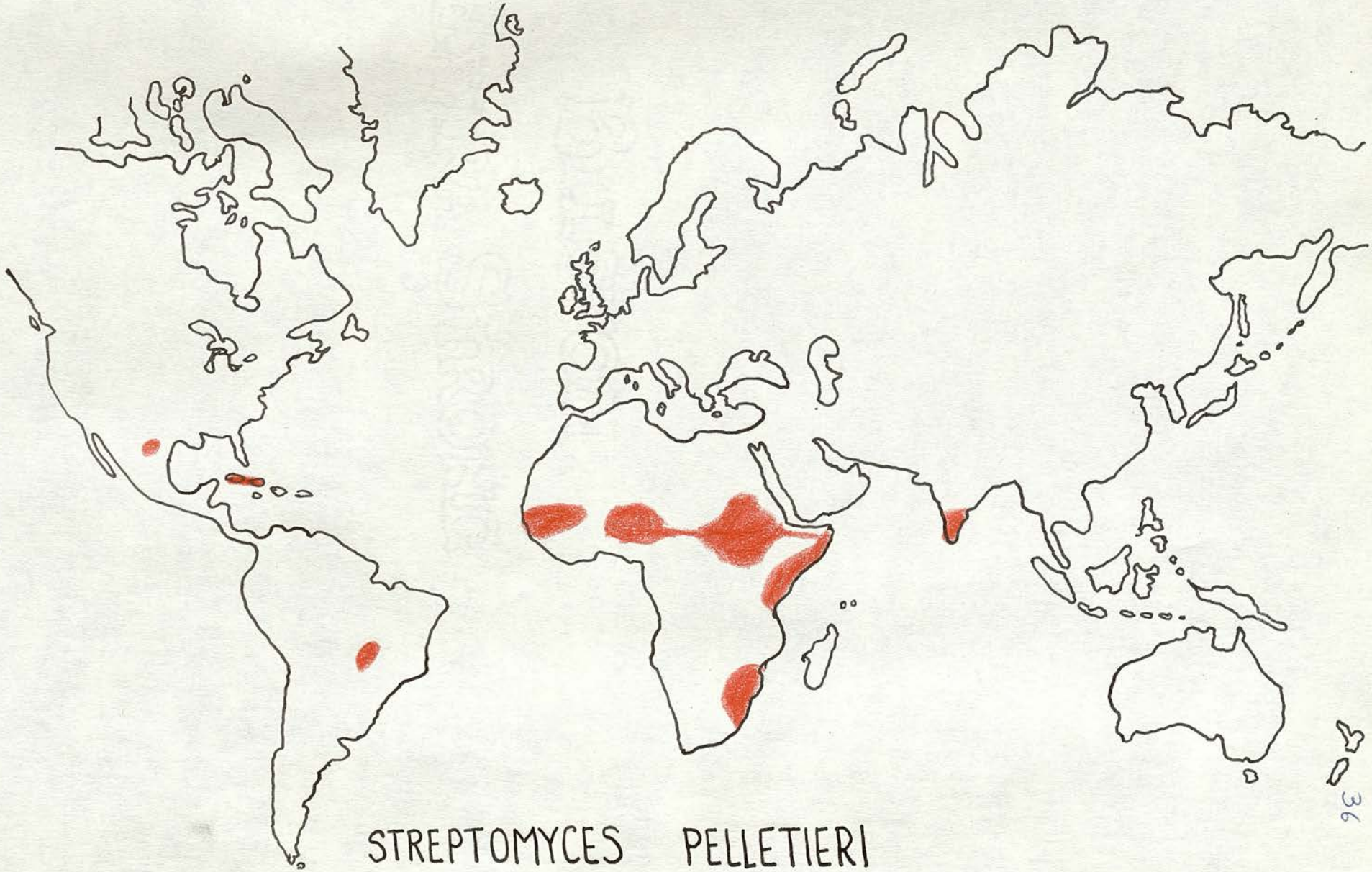


MADURELLA MYCETOMI


PROBABLE CASE
MADURELLA GRISEA
PHIALAPHORA JEANSELMEI


ALLESCHERIA BOYDII
Human isolates.
Soil isolates.


DOUBTFUL CASES
GENUS CEPHALOSPORIUM
STREPTOMYCES SOMALIENSIS



STREPTOMYCES PELLETIERI

NORMAL HABITAT OF THE SPECIES.

Little is known of the normal habitat of these organisms as only two species have been found outside the human body. It seems probable that most exist on soil and vegetable matter.

Allescheria boydii has been recovered from soil samples in Tennesee, Maryland and a chicken run in Panama (Ajello 1952 - 1954). This fungus has also been observed as a contaminant introduced by a lumbar puncture needle during a spinal anaesthetic. This tragedy which occurred in the West Indies, was investigated but the source of the contaminant was never elucidated (Aronson et al).

Nocardia asteroides was isolated from soil samples by Gordon and Hagan in 1946 and again by Emmons in 1949.

The genus Streptomyces is widely distributed in nature, most species existing as saprophytes in the soil and on plants or decaying vegetable matter, but pathogenic strains have not been included amongst the isolates.

The saprophytic varieties of Cephalosporium which are so ubiquitous in the air and cause contamination of cultures are not identical with the strains causing mycetoma.

Phialaphora jeanselmei is probably related to certain wood fungi.

A number of workers have attempted unsuccessfully to isolate the organisms from the soil. One has only to read the papers of Ajello and Gordon and Hagan to realise the many technical difficulties that face any investigator of this branch of mycology.

Negative findings do not therefore necessarily imply that the fungi are not present in the soil as our techniques may not be sufficiently refined.

GROWTH OF ORGANISM ON SOIL AND PLANTS.

Though direct search for the organism have usually been unavailing, the converse experiment of growing cultures of the organisms on soil media and on living and sterile thorns have been attended with better results.

A soil filtrate media in a jar containing no additional protein or energy sources has been shown to support both the "white actinomycetes" (? Streptomyces madurae) and the black Madurella mycetomi. (Vasudevan et al. 1929) Abbott grew Madurella mycetomi in Sudan cotton soil. He observed that with this starvation medium aleurospores developed, and suggested that these bodies may be the form infective to man. This is possible but unlikely as such bodies do not arise with other species. Low energy media may however, modify the fungus and reveal the form in which it is most prevalent in nature. Thus, Monosporium apiospermum on such a media occasionally develops ascospores and enters its perfect sexual stage of Allescheria boydii (Creitz & Harris 1955). Experimental work does not suggest that there is any difference in the pathogenicity of the two forms of this specie.

Streptomyces pelletieri and a "white actinomycete" have been cultured on sterilised bamboo and prickly pear thorns. (Vasudevan et al 1929). Madurella grisea has been grown on a cactus by Mackinnon who noted different forms and rates of growth according to the seasons. During dry periods the mycelium was scarcely visible and quiescent, but upon the onset of more favourable humid conditions activity again became apparent.

HARDINESS OF GRAINS.

The grains discharged from lesions may be viable for long periods under apparently most unfavourable conditions such as transmission by post in the dried state. Madurella mycetomi grains have been shown, by eventual culture, to have survived more than 120 days at the ambient temperature of the Sudan sand (Abbott 1953). Streptomyces madurae successfully resisted nine months on sterile dry blotting paper (Vincent 1894). Sunlight per se does not appear to be inimical to the survival of grains. (Abbott) This particular finding may have a bearing on the natural history of the disease.

In primitive rural areas, far from medical care a patient may delay many years before seeking treatment. Many hundreds of grains may have been discharged during this period leading to a high local concentration of the organism in the soil near his dwelling and workplace. The grains can survive, and indeed may grow, on the soil so that around a case there is possibly a focus for infecting further cases. It is difficult to prove transmission of this type in an individual case but this mechanism may well operate in areas of high incidence. In the Northern part of the Sudan 300 new cases occur annually but in the adjoining Nile valley the condition is rare. The effect of the annual flood of the Nile which flushes and renews the soil may perhaps be held accountable for the difference in the prevalence of the disease in the two neighbouring areas. Moisture itself does not seem to favour mycetoma, as it is rare in areas of high rainfall or where fields are frequently under irrigation.

SECTION IV.

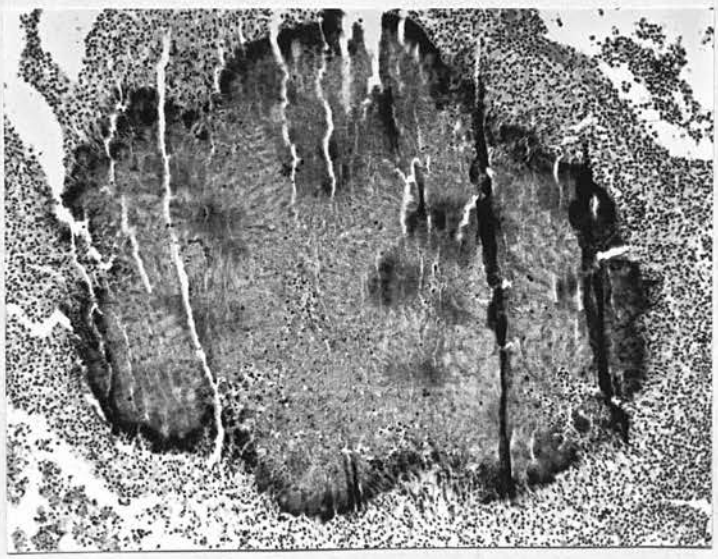
GRAINS.

GRAINS.

The discharge of grains from sinuses is the best known sign of mycetoma but it is not a pathognomic feature since it can also occur in other diseases.

Actinomycosis due to A. hominis and A. bovis is frequently associated with yellow 'sulphur' granules in the discharges. Black grains are not infrequent in aspergillosis, particularly the non-pulmonary forms. Very small black grains have been observed in chromoblastomycosis due to Phialaphora sp.

All the above diseases differ clinically from Madura foot, but in cases of doubt cultural studies would furnish the diagnosis.



Grain of Actinomyces hominis from a hepatic abscess.

From the cases illustrated in the literature A. bovis and A.hominis are pleomorphic in their parasitic stage. I have not studied sufficient examples to know whether these species of anaerobic organisms can invariably be differentiated from each other or from the aerobic organisms.

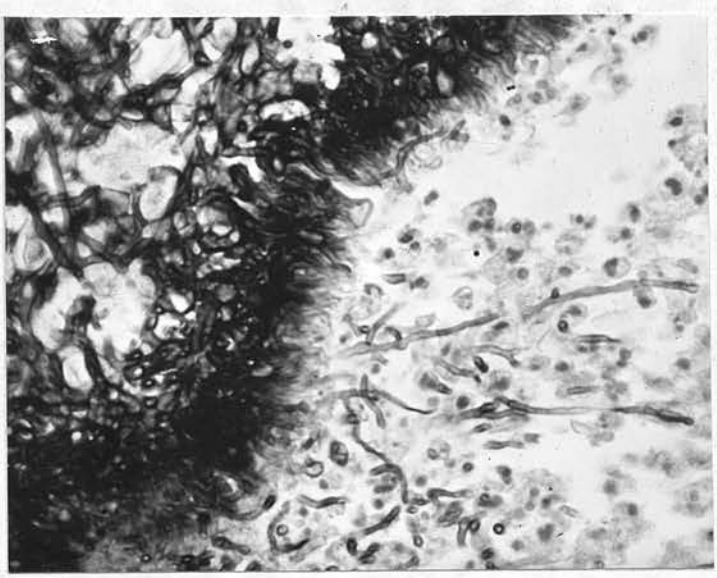
ABSENCE OF GRAINS IN MYCETOMA.

Grains are not always seen in true mycetoma. Small yellow or white granules are very difficult to see and can be readily missed unless pus is examined microscopically. This error is unlikely to occur in the black maduromycoses and Streptomyces Pelletieri infections, where the colour of a grain is of sufficient contrast for itto be immediately apparent.

Grains cannot be discharged when there are no sinuses, though they may be present in the depths of the lesion. This is noted particularly in certain clinical forms of Madurella mycetomi infections where closed nodular lesions containing grains exist in the soft tissues or even deep in bone. The grains eventually appear with the development of the sinuses but this may be only a relatively late feature.

Very rarely grains may not be evident at all, either externally or in the tissues, though clinically the lesion is otherwise typical and culture of pus has resulted in growth of a mycetoma pathogen. This most frequently occurs in Nocardia asteroides lesions. Biopsy of such a case may reveal, perhaps only after diligent searching, a few fine gram positive filaments. These may merely show slight branching or may be more conglomerate and tangled. They are not true grains as the fragments are not actually linked together in any way. A similar absence of grains has been observed with Nocardia braziliensis, particularly in systematised Nocardiosis which is not strictly a mycetoma. Nocardial infections in which the precise specie has not been established have been reported with the absence of grains. (Panja 1955). This form of behaviour has also been noted in classical actinomycosis.

In a case of Madurella grisea mycetoma studied in Venezuela no grains were ever seen clinically nor could any be demonstrated on histological examination. Sections showed areas of scattered



MADURELLA GRISEA
(Hyphae spreading from grain)

Mycelium is here seen to be extending beyond the confines of a grain.of Madurella grisea. The walls of the hyphae are well seen through the use of Schiff periodic staining technique.

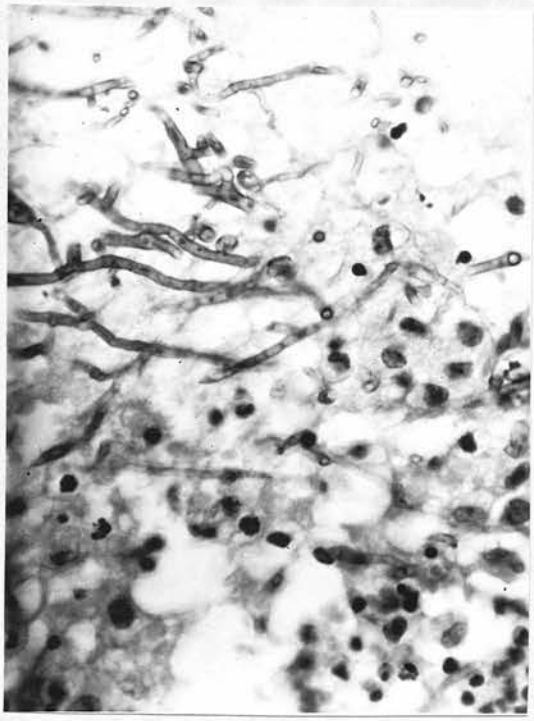
Enlargement : 600. Case of Kanthack received in the museum of St. Bartholomew's Hospital in 1884.

Fig 4

hyphae and a few longer lengths of mycelium. Culture was positive (Mackinnon et al 1954). Figure 4 shows the edge of a grain attributed to Madurella grisea with mycelium extending into the tissues. This would appear to be a lesser form of the same process. The only other case I can find of this type is that of Monosporiosis studied by Reifferscheid and Seeliger (1955). In their patient true granules were seen but histological preparations showed extension of mycelium into granulation tissue.

A total absence of granules does not exclude a diagnosis of mycetoma. There have been a few instances in which an organism has been cultured but no parasitic forms could be observed even following examination of the amputation specimen.

Shortly before the last war a London cabinet maker was admitted to St. Bartholomew's Hospital (No. 145069) under the care of Mr. J.P. Hosford. The patient had noted swelling and pigmentation of the foot for 6 years. An ulcer had been present on the foot for 6 months prior to admission and the femoral lymph glands were found to be enlarged. In addition there was a partial palsy of the sciatic nerve with sensory loss. X-rays showed two type of bone changes. Firstly, trophic bone changes of the type associated with leprosy, tabes and diabetic neuropathy. Presumably these disturbances were related to the sensory disorder which was not fully investigated. Secondly, there were multiple foci of osteitis and periostitis, and a periostitis also involved the lower ends of the tibia and fibula. On two occasions 'Actinomyces madurae' was cultured. From the notes it is not possible to determine whether this identification refers to a Nocardia or to a Streptomyces madurae. Filaments were found in the pus but no grains were ever detected. I removed tissues from the preserved amputation specimen, but found no trace of grains after examining sections from several blocks. This case, whilst not a typical



Ramifying septate hyphae lying loosely near the ostium of a sinus. This exceptional occurence was found in the case of Monosporiosis studied by Seeliger. Other sections revealed typical grains of this specie.
Photomicrograph by courtesy of Dr. Seeliger.

Fig I

mycetoma, would seem to be the only example of the condition ever to have occurred in this country.

In the U.S.A. a case of Paget's disease developed a Streptomyces madurae infection at the site of a fracture. No grains could be found. The source of infection is unknown but as the fracture was not compound a haematogenous aetiology is most likely. (Thompson and Vernon Wax 1950).

These last two cases would once have been termed paramycetoma but nowadays this term seems no longer to be used. Two cases of this nature recently reported from the Honduras were probably mycetoma even though no grains were found (Schapiro 1954-1955).

'Mossy foot' is another non specific label that has also largely lapsed. Since the term lacks precision, such varying conditions as filarial lymph-oedema, chromoblastomycosis, crab yaws, sporotrichosis and Madura foot have been included under the label.

STAINING REACTIONS.

In a doubtful case the correct staining of tissue sections will greatly facilitate the detection of parasitic elements.

The fungi imperfecti are best revealed with a periodic stain such as Schiff's reagent which is taken up by the walls of the hyphae. This reagent is considered to act on the mucopolysaccharides within the hyphae by oxidising hydroxyl groups to aldehydes which then stain. Therefore there is a lack of specificity as surrounding structures containing glycogen, starch, cellulose, mucin and mucoprotein are similarly hydrolysed and oxidised.

In practice confusion is unlikely when looking for fungal elements. Though there are many modifications of technique available, I have found that the best contrast is provided by a 0°1% neutral green counter stain.

Weigerts modification of Gram's stain is excellent

for the demonstration of Actinomyces. Mackinnon (1954) believed that it might be possible to determine whether a Nocardia or Streptomyces was present in the tissues by the use of a semi-acid fast staining reaction. The value of this test is limited as I have found it negative in known Nocardial braziliensis infections.

The converse finding - that acid fast grains indicate a Nocardia - is probably valid. The technique that I have used, at Mackinnon's suggestion, is decolourisation by 1% sulphuric acid for five minutes. Any method in which decolourisation is not too vigorous would probably do equally well. Plant's stain for the Actinobacillus provides a good contrast, red parasite against a yellow picric acid background, but it is not a reliable test of acid fastness. With the above reservation it is a useful stain for studying grains though the histological patterns are then less obvious.

DIMORPHISM.

The grain and the cultures of the maduromycoses are both recognisably mycelial and do not show any dimorphism. This is in marked contrast to such systemic mycoses as coccidiodomycosis, torulosis, histoplasmosis and paracoccidiosis braziliensis where the parasitic stage is yeast-like bearing no resemblance to the cultural form. This phenomenon is also a feature noted in some plant pathogens which on artificial culture media are very different from the mycelial parasitic stage.

NATURE OF GRAINS.

Essentially a grain represents a colony of the organism. A colony free to expand during growth in all directions assumes a spherical form. This is well seen, for example, in shake deep broth cultures of Actinomyces hominis. The classic 'puff ball' colonies are seen dispersed through the liquid.

This form of growth is more difficult to elicit in the aerobes which do not readily grow beneath the surface. Yazbek (1920) managed to obtain such a preparation with Madurella mycetomi.

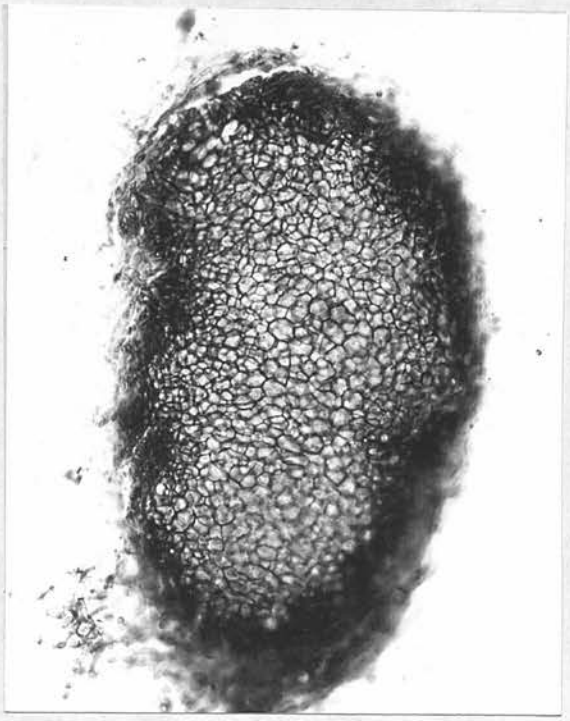
Unlike most bacterial colonies which fragment or disperse in the body, the mycetoma organisms tend to remain compact and discrete. This generalisation does not always apply to the Nocardial infections.

The mode of growth of a grain by active peripheral expansion may lead to an amorphous, perhaps dead, central portion. Presumably this area becomes inadequately supplied with nutriment due to its distance from the surface of the grain. Streptomyces somaliensis invariably shows a central amorphous area. In larger grains of Streptomyces madurae this also occurs and may proceed to apparent cavitation. Such phenomena are rarer in the hyphomycetes which are more highly organised.

Most species, Streptomyces pelletieri being a notable exception, owe their characterisitic radiate appearance to the branching of the tips of the peripheral elements of the colony.

Nocardial and Streptomyces madurae grains frequently show peripheral 'clubs'. These however, never occur in cultures. The nature of these club like excrescences is unknown. It has variously been suggested that they may be related to a host-parasite sensitivity reaction or alternatively that they form part of a defensive action to an unfavourable environment. Most explanations are more teleological than scientific.

The expanded processes seen at the periphery of fungi imperfecti grains are of an entirely different nature. These are the chlamydospores that are also present in cultures of the species. These specialised structures, larger than hyphae, contain cystoplasm loaded with food material. Chlamydospores are as a rule most frequent at the periphery of a grain but they are found centrally frequently in tissue forms



Section through a sclerotia of Madurella mycetomi. The characteristic central polygonal cells contrast with the periphery composed of fuliginous hyphae. The difficulties encountered in embedding and cutting such small and friable structures has led to a section thicker than the standard histological preparations. Sclerotia of Allescheria boydii are very similar in appearance. (No stain x 275)

Fig 6.

of Allescheria boydii and Madurella mycetomi. When many chlamydospores are present within a granule a vacuolated appearance is seen on section.

Mackinnon (1954a) considers such grains to represent 'sclerotia'. I feel that this view is mistaken as sclerotia are entirely different in nature.

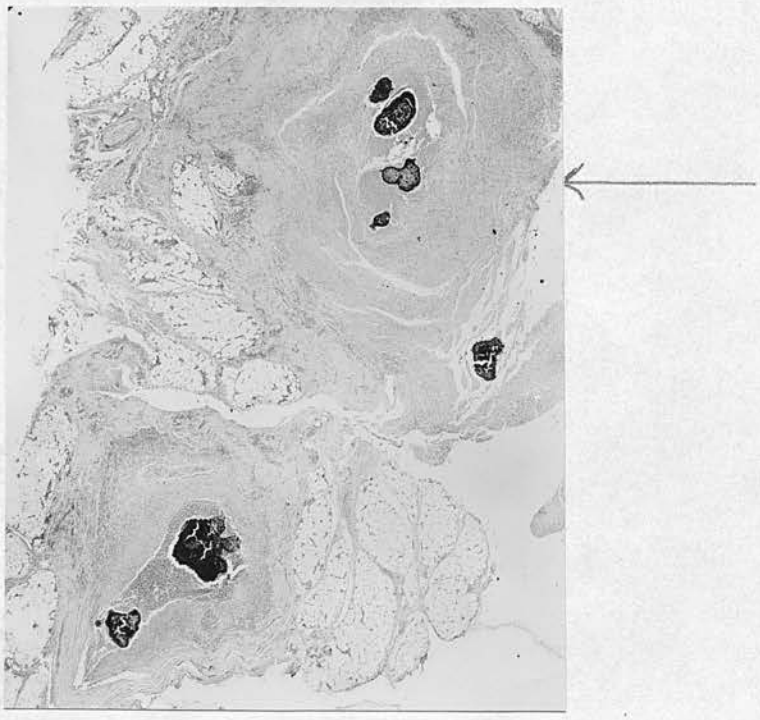
SCLEROTIA.

Sclerotia are inconstantly occurring bodies which develop on culture as storehouses of reserve materials by which the organism can survive adverse conditions. Sclerotia usually become separated from the main mass of the colony and can remain dormant for considerable periods. Ultimately the energy reserves are expended in the production of germinating shoots thus differing from chlamydospores.

Sclerotia that arise on cultures appear, as a rule, as small pigmented bodies above the surface of the aerial mycelium. On section the periphery is cutinised and felted by fuliginous hyphae whilst the central portion consists of numerous polygonal cells containing the food depots.

A sclerotia can grow and develop where nourishment is lacking but a grain of mycetoma remains inert until placed on a suitable medium. An ordinary vegetative mycelial grain cannot give rise directly to fruiting structures whereas a sclerotia is able to. A sclerotia is only a part of a colony that has differentiated for a particular purpose.

Mackinnon's suggestion is based only on a superficial similarity on section between certain grains and true sclerotia. However, as Madurella mycetomi grains are very polymorphic and show no sharp division between vegetative and 'sclerotoid' forms (an intermediate vacuolated type is common), the analogy is not valid. In a single biopsy several different types of grains may be seen as in the illustrations overleaf.



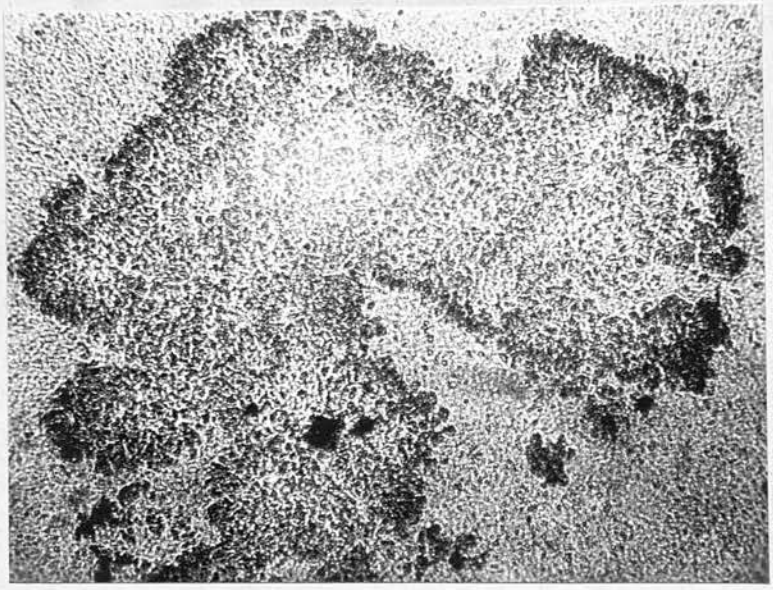
This single low power field reveals several different types of grains imbedded in fibrous tissue ; demonstrating the polymorphism that exists in the parasitic forms of Madurella mycetomi. The differences in appearance are accounted for by the number and position of chlamydospores. The arrow indicates a grain of the type which Mackinnon terms sclerotia. (Haematoxylin and oesin- ×17)

Fig 7.

MYCOLOGICAL IDENTIFICATION BY GRAINS.

It is a fundamental tenet of the biological sciences that each specie, though subject to a range of variation, can be recognised by its morphology. The identification may not be precise, but at least it should be possible to determine the genus of the organism. Amongst closely related species clear differentiation is not always possible by morphology alone as the distinctive attributes may only be elicited by biochemical or other non-visual methods. The organisms responsible for mycetoma can be readily recognised in their saprophytic stage in the laboratory on the basis of morphology and staining reactions but it does not follow that similar methods will suffice to distinguish the various organisms in their parasitic phase. For a variety of reasons, the chief being the limited experience of any individual investigator, few attempts have been made to relate the structure of grains as seen in histological preparations to the causative specie. Several workers living in endemic areas and who have had the opportunity of observing several cases due to the same specie, have noted a similarity in the forms of grains from case to case. Mackinnon, in an address to the Royal Society of Tropical Medicine and Hygiene, stated that the parasitic forms of the black maduromycoses were morphologically distinct and capable of being identified in histological preparations. He has suggested to me that this might also apply to the other species including the actinomycetes. He kindly made available to me some preliminary notes on this subject. I have investigated this problem in detail and have found cause to amend some of his views, which were based on limited material.

The methods employed in this study have been firstly to review the literature and then to obtain numerous sections from all over the world. I was fortunate in obtaining the co-operation of many workers who



Cover slip preparation of a crushed granule of Monosporium apiospermum surrounded by exudate and debris. This picture reveals the difficulties of interpreting a wet film. All that can be determined is that a maduromycete is present which may be either a member of the genus Cephalosporium or ,as in fact is the case, an Allescheria. It must be admitted however that clear differentiation between these two possibilities cannot invariably be made even with a good histological section.

Fig 8

gifted or loaned material from their personally collected cases. In this way I have been able to study several examples of each specie except for the very rarely occurring Phialaphora jeanselmei, of which I have one culturally proved instance and Nocardia asteroides of which I possess only a probable case. Even more significant has been the inclusion of some thirty or so biopsies containing granules derived from cases where no cultures had been obtained. If the proposed scheme of classification of grains which I shall describe is valid it should now be possible to identify in retrospect the organisms responsible. In fact, with the exception of a few sections which can only be provisionally placed and one totally unidentifiable case, the others have all been readily labelled. Amongst the limitations that apply to the method is the possible existence of as yet undescribed species causing mycetoma.

The details of the grains of each individual specie are described in their appropriate sections in the second half of this thesis. All the species show a degree of polymorphism but there appear to be sufficient stable characteristics to particularise some specific features of each.

It is necessary first of all to decide whether one is dealing with an imperfect fungus or an actinomycete. This is achieved by noting whether the mycelium is composed of septate hyphae as in the maduromycetes or of fine filaments, less than 1 μ in diameter as in the actinomycetes.

The black maduromycetes are distinguished in the following manner. Madurella mycetomi shows hyphae whose walls and interior contain brown pigment. In addition the hyphae are separated from each other by an amorphous, brown interstitial cement substance. Madurella grisea grains reveal a central, unpigmented ramifying mycelium with a darkly pigmented peripheral zone of chlamydospores. Phialaphora jeanselmei has

hyphae with brown walls but no interstitial pigmented cement. There are other distinguishing characteristics that could be applied but the preceding are the most important.

The pale maduromycetes that need to be distinguished are Allescheria boydii and members of the genus Cephalosporium. The former possesses thicker hyphae which are more radiate in arrangement and may be intermingled with chlamydospores centrally as well as at the periphery, whilst the latter show smaller hyphae disposed in a whorled pattern and chlamydospores appear to be confined to the periphery. The Streptomycetes are readily separated from each other. Streptomyces pelletieri is immediately obvious on an account of the red or dark orange colour of the grains as seen by the naked eye. On section these are lobulated or reniform with a well defined periphery containing Gram positive fine, branching filaments and coccal bodies. The grains of Streptomyces somaliensis tend to be oval with a well defined periphery and, as a rule, a completely amorphous internal structure. A careful search may disclose a few grains which possess an internal ring of fine Gram positive filaments extending radially near but not right up to the periphery. Streptomyces madurae grains are very variable in shape. Centrally there are Gram positive filaments and spores which condense at the margins to a thick mantle from which extend clubs or, more frequently, an eosinophilic, hyaline substance.

The Nocardia require the use of a semi-acid fast staining technique to help in their identification. The grains of Nocardia brasiliensis are lobulated and contain central Gram positive filaments, embedded in an amorphous matrix which is only faintly dyed. Clubs are occasionally seen at the periphery but it is more frequent to find merely a structureless margin which shows semi-acid fastness. Nocardia asteroides grains are very rarely found, as a tangled

mass of branching filaments is the only evidence of the organism to be found in the tissues. These filaments show variable degrees of acid-fastness. True grains have been reported, the small ones consist of radially disposed acid-fast filaments with little central structure. Clubs may occur in the larger grains.

The grains of anaerobic classical actinomycosis that I have studied are very polymorphic and I find it difficult to see any constant pattern in their structure. Therefore, I cannot state definitely whether or not they are liable to be confused with any of the preceding species.

The reliability of this key to specie identification can only be established by examining further biopsies from cases where the specie has been identified on culture and determining whether the two methods of diagnosis agree. Limitations are obviously inherent in the technique as several species that may cause mycetoma still await identification. Lastly, it must be emphasized that several grains should be studied from each case as so much polymorphism exists in some of the species.

The diagnosis of Madurella mycetomi, Madurella grisea and the Streptomyces is easy but to distinguish Allescheria boydii and a Cephalosporium is more difficult and uncertain as is the differentiation of the Nocardia. I hope that the features described will prove sufficient.

Should this method prove valid its main importance will be in the provision of a more rapid and more readily available means of determining the offending agent. Specie determination will probably assume increasing importance once chemo-therapeutic substances are found to exert specific action upon particular species. This state of affairs has already been attained with several species.

SECTION V.

AETIOLOGICAL CONSIDERATIONS.

AETIOLOGICAL CONSIDERATIONS.

'Parasitic life is only an accident in the life of a saprophyte' is an aphorism that bears particular relevance to mycetoma (Ainsworth 1952). We are ignorant as to how the accident occurs in this condition. We are uncertain both of the normal habitat of the saprophyte and of the form which is infective to man. I know of no data which would determine whether the vegetative mycelium, the conidia, aleurospores, ascospores or sclerotiae are infective. Animal inoculation experiments throw little light on this as the methods that have been employed are not strictly comparable with those obtaining outside the laboratory.

Undoubtedly the usual method of infection is entry of some fungal element through an abrasion or by direct implantation by a penetrating wound into the tissues. The prevalence of the disease in the foot is to be expected amongst people who do not wear shoes and frequently injure themselves in that region. Indeed trauma to the foot is such a prosaic occurrence that, unless severe, an individual incident is not notable. For this reason it is as a rule difficult to calculate later when the infection might have been contracted unless the site involved is unusual.

LATENT INCUBATION.

In the Senegal it would appear that the usual method of infection is through a deep wound of the foot inflicted during the annual tilling of the fields. In several such cases the incubation has been calculated at between three and six months. (Champeau 1950).

A further history in which it is possible to relate a specific trauma with the development of clinically obvious lesions is given by Grantham Hill (1931 Case E - 143). A youth fell off a camel into a spiky bush with the result that many thorns penetrated the sole and heel of his right foot.

These were plucked out at the time and no suppuration ensued. Two months later several nodules appeared at the site of entry of some of the thorns. At operation four separate black (Madurella mycetomi) mycetoma were removed and three of these contained thorn fragments.

These examples show that the period between infection and gross evidence of the disease may be as short as two months or so. However, from the study of my own cases and others I feel that the latent period before signs are manifest depends a great deal on the site of inoculation. The examples cited above resulted in superficial lesions which were readily apparent.

ROLE OF THORNS.

Thorns have been blamed for causing the disease since the days of Carter. In 1870 Holmsted of Hyderabad described a thorn he had removed from the midst of a black mycetoma. (Carter 1874).

Fragments of thorn are frequently found in the foot of Arabs in the Aden Protectorate. On reflecting skin when carrying out orthopaedic procedures it is not uncommon to find such material deeply embedded in fibrous tissues. Grantham Hill found thorns in 30% of the mycetomas in which he had operated.

This high incidence, though it is not conclusive, strongly suggests that thorns provide a portal of infection. It should also be remembered that most of the spikes of plants found in arid tropical regions are strong and do not readily break off. Most are plucked out without leaving any parts in the tissues.

Smith (1955) of the Sheikh Othman Hospital, Aden, found thorn particles in a mycetoma involving the shoulder. In such a position a causal relationship is more likely as thorns injuries are unusual in that region.

Though I have found thorns as foreign bodies in

orthopaedic patients only once have I observed them in a mycetoma. This was of the superficial plantar type due to Madurella mycetomi (illustrated on page 74)

FURTHER MODES OF INFECTION.

Thorn wounds and abrasions cause most of the cases. There are on record several more unusual modes of infection that have been incriminated. The most bizarre followed four months after a cobra bite. (Case 5 Yazbek 1920). There have been three alleged cases where the organism entered the body through the exit hole of a guinea worm. Rustomji (1859) and Bonrepeaux (1924) each observed such a patient. Noc and Jouenne (1922) dealt with an unusual case. A father and son slept together on the same couch. The father had a chronic discharging black grain mycetoma. The son developed a Madurella mycetomi lesion in his leg at an opening from which a guinea worm had been extracted in the native manner.

I have found only a single instance of a lesion arising from a tropical ulcer. This is a common lesion in the tropics but rarely does mycetoma ensue. A European developed a tropical ulcer when working in the Congo. This responded to therapy, including aureomycin, but before healing was complete nodules and fistulae formed and a Nocardia was isolated. (Gate et al. 1952). As this is the sole example on record of this type it is tempting to assume that the broad spectrum antibiotic may have altered the bacterial flora so that a fungus could obtain a foothold.

A Nocardial granuloma of the forearm followed a hypodermic infection given in India (Cullen & Sharp 1951). The meningeal infection by A. boydii which resulted from a lumbar puncture has been already mentioned.

Splinters from a plank caused a Phialaphora jeanselmei mycetoma in New York City (Symmers & Sporer

1944).

Though I have been unable to obtain exact details Doctor Petrie has told me of a Theatre Sister in Aden who developed a mycetoma on her thumb several months after accidentally puncturing it during a mycetoma operation.

PATHOGENESIS OF ALLESCHERIAL INFECTIONS.

Most cases of monosporiosis have been reported from temperate climates and have been well investigated. Most of those affected have habitually worn shoes and because of this, in the majority of cases, it is possible to relate the origin to a specific trauma. In nearly every instance a cut, abrasion or compound fracture seems to have provided the means of entry for the fungus. An example of this and the chronic nature of the malady is the case of Shaw (1935) which was diagnosed thirty-two years after a laceration caused by a wagon wheel. The recently reported patient of Reifferscheid and Seeliger (1955) had been ill for twenty-six years before the true nature of the infection was determined. The gluteal region was involved in this instance following a fall in a privet hedge. As Allescheria boydii has been found in the soil on diverse occasions it is not surprising that nearly all the patients have been agricultural workers.

FAMILIAL FACTORS.

Exceptionally mycetoma has been present in several members of a family. This does not indicate any inherited predisposition but only demonstrates that near association with a carrier naturally increases the chances of infection.

The case of Pinoy in which a father and son were both infected has been alluded to already. A Yemeni of the Dayani tribe reported to the Aden Protectorate Levy Hospital with a black grain mycetoma of one months duration. He stated that his father had had his foot amputated for a like condition several years

previously. Familial involvement appears in this instance to have been coincidental. The interest is rather in the rapidity with which the son sought medical aid. Fortunately a happy ending resulted as a simple local excision followed by a skin graft effected a cure.

A more startling coincidence was the unfortunate occurrence in a Sudanese woman of a Madurella mycetomi lesion of her right foot and a Nocardial mycetoma of her right wrist. (Abbott 1954).

Sartory, Meyer et al. (1930) have given details of mycetomatous-like Hemispora stellata infections which affected the legs of three Parisian brothers. This case is discussed in further detail later.

REASONS FOR THE RARITY OF MYCETOMA.

There is no convincing explanation to account fully for the rarity of the disease if it be accepted that infection follows merely a breach in the skin.

It is not possible to verify the most simple explanation which is that the causative organisms, whatever their habitat, are very sparsely distributed in nature. This theory probably explains the rarity of mycetoma in certain climates where the fungus cannot thrive and in addition shoes protect the inhabitants.

It seems that the introduction of pyogenic organisms at the same time as the fungus renders mycetoma less likely. Bacteria grow more rapidly and overwhelm the fungus before it can become established in the host. This may be due to the more rapid adaption and utilisation of metabolites by bacteria but, probably, a more important mechanism is the inhibitory effect of bacterial enzymes and dying cells. The clinical history of cases frequently reveals the absence of sepsis in early stages but this is by no means invariable. In Senegalese patients inflammation is frequently marked owing to the local practice of applying dung to all wounds (Champeau 1948).

Repeated inoculations of the organism may be required before the parasitic stage can become established. This, if so, would be analgous to leprosy in which it is believed that a single chance inoculation is not sufficient but that a repeated long-continued association is required. Perhaps in this context it is noteworthy that some of the acid-fast Nocardia are closely related to the mycobacteria. Actinomycosis, with typical granules, has been induced in guinea pigs by repeated inoculations when a single or a few proved of no avail. (Slack quoted by Henrici 1940) Presumably the repeated presence of the actinomycete in the tissues led to a change in the resistance of the host. This increase in susceptibility of the tissues may be due to the gradual development of hypersensitivity to the organism, or its products, causing eventual cell destruction. This form of 'autocatalytic' phenomenon (Henrici) is characteristic of the deep mycoses, as distinct from the localised and self limiting superficial mycoses. In behaviour mycetoma is closer to the deep mycoses though the tendency to remain localised is not in common with the majority of such mycoses.

HYPERSENSITIVITY.

In all the deep mycoses adequately studied hypersensitivity has been shown to occur. Frequently the development is slow, perhaps because the thick wall of fungal cells only allows the diffusion of antigenic intracellular protein at a slow rate. (Henrici 1940) Sensitivity is not always highly specific but it has been considered to be adequate for epidiemological surveys. For the latter purpose as well as a diagnostic aid skin tests have been evolved particularly for coccidioiodomycosis and histoplasmosis. With respect to mycetoma data is scant. When attempts have been made to elicit allergy the results have usually been negative.

Seeliger (1955) a mycologist of Bonn, has been making

a particular study of cutaneous sensitivity reactions, complement fixation tests, and precipitation tests, with immune sera. His results have not been published as yet but he has given me some information concerning them. The techniques of preparing his reagents are somewhat technical and as I have not full details I will merely report some of his findings. In his case of monosporiosis all the tests were positive and highly specific. He investigated controls with negative results and preparations derived from a variety of other fungi imperfecta did not induce a positive reaction in the patient. Dr. Neuhauser (1955a) of Chicago informs me that Dr. Seeliger was able to demonstrate specific antigens in the serum of her case of Madurella grisea mycetoma.

Nino (1941) found that his patient with monosporiosis was sensitive to an extract of spores of this organism.

Serological tests have proved positive in Nocardial infections though this has been so, most often in disseminate nocardiosis rather than in localised granulomatous lesions.

Experimentally acid fast nocardia can show a cross immunity with the Tubercle bacillus suggesting a common antigen. False positive reactions with tuberculin have been reported also. (Ludwig & Hutchison 1949).

The present state of knowledge indicates, if nothing else, that the tests that have been devised are likely to remain academic exercises. It is doubtful if aids of this nature, even if highly specific, will be of great clinical value.

SECTION VI.

THE CLINICO - PATHOLOGY OF MYCETOMA.

THE CLINICO-PATHOLOGY OF MYCETOMA.

"Infectious diseases present specific earmarks which are determined by the species of microbes which cause them; nosology reflects taxonomy.

Henrici 1940.

The term mycetoma or Madura foot is applied to a clinical entity which may be caused by any one of a number of organisms belonging to several genera and species. With such a wide spectrum of infective agents, differences in the pathological manifestations which may be encountered are to be expected, but these do not appear to have been investigated except in respect of two species. In the Sudan, where the condition is common, workers have observed certain differences between the pathological changes caused by Streptomyces somaliensis and Madurella mycetomi (Grantham-Hill 1931, Abbott 1954).

My own clinical impression, borne out by references to the literature, has been that whereas some separate species can be found to manifest themselves quite differently from one another in the course of infections, yet it is not the case that all species are clearly identifiable on the basis of their clinical and pathological manifestations. In respect particularly of the more rarely occurring species there has so far been insufficient information accumulated to observe whether or not they conform to a constant pathological pattern. But whilst no rigid classification is possible and whilst it is likewise impossible to arrange the organisms in order of ascending virulence it is, I think, permissible to claim that Nocardia asteroides and Streptomyces pelletieri are responsible for the most severe manifestations of the disease. It is, of course, necessarily difficult to gauge the relative severity of cases in a chronic disease

where death is very rarely directly attributable to the infection.

I have come to the conclusion that there are four main factors at work which combine to determine the final nature of the pathological changes in any individual case.

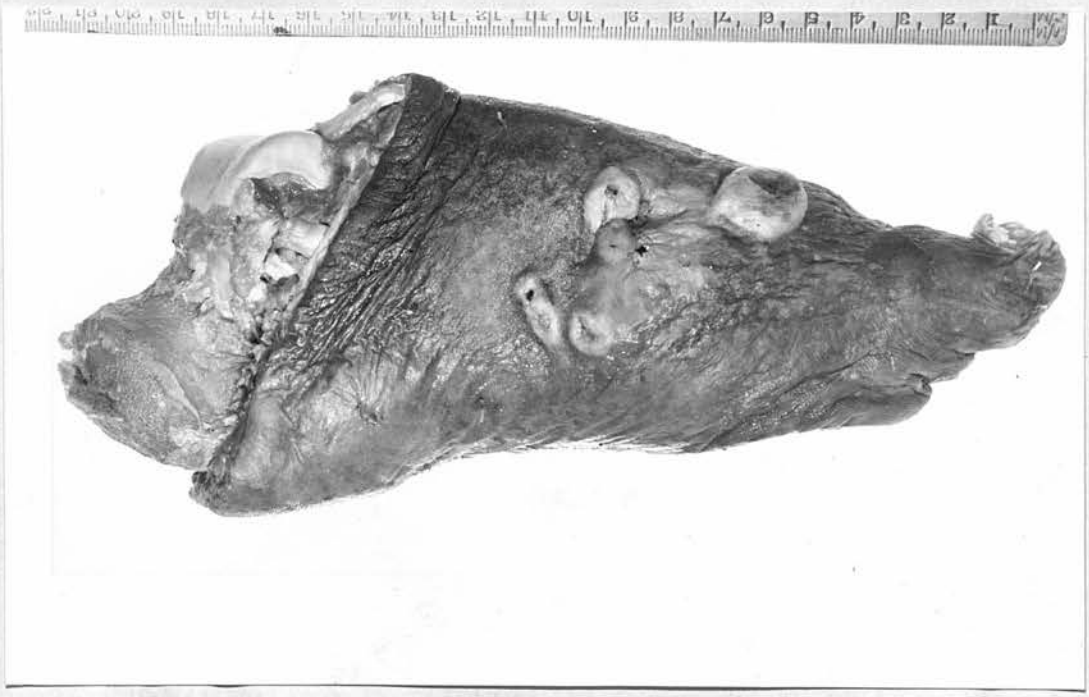
A. The specie responsible for the infection is of particular importance during the early stages of the disease. Thus, for example, lesions due to Madurella mycetomi are more bland than those due to Allescheria boydii and Madurella grisea infiltrates to a lesser extent than Streptomyces pelletieri.

B. The original site of implantation of the organism is largely responsible for the form the disease will take. This is a factor of particular relevance to Madurella mycetomi infections.

C. The presence or absence of secondary pyogenic organisms within the affected tissues has a great bearing on the subsequent progression of the disease.

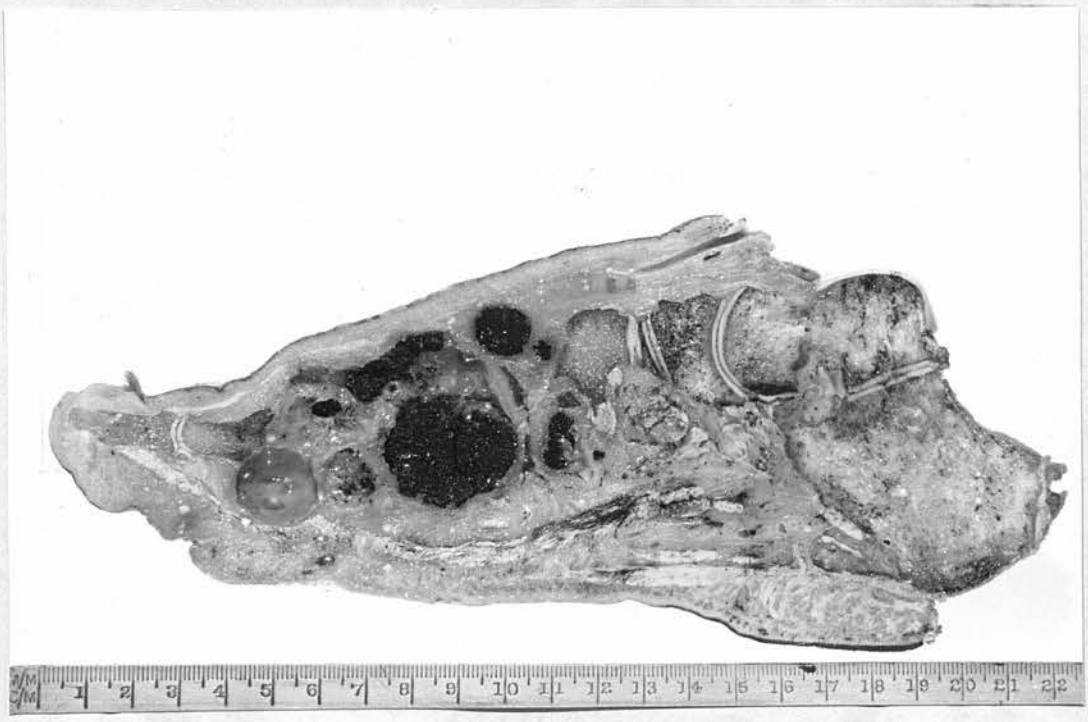
D. Finally, it is the duration of the disease which is of the ultimate importance in any consideration of the changes to be found in mycetoma. Although the earliest manifestations are undoubtedly conditioned by the three factors mentioned above yet, with the passage of time, these exert less and less influence upon the pathological processes which have been set in motion, so that the final result is likely to be similar in a whole range of infections. A maduromycosis will be indistinguishable from an actinomycosis, a lesion originating in the subcutaneous tissues of the dorsum of the foot may resemble one arising deep in the os calcis and a primarily sterile Madurella parasitismmay have little to distinguish it from an initially pyogenically invaded Nocardial wound.

Since my own clinical experience has been largely with Madurella mycetomi infections I propose to



Foot removed by Syme type amputation showing the orificesof several sinuses at the summit of fibrous pale nodules. A black Madurella mycetomi grain can be vaguely discerned at the mouth of one of the sinuses. This is one of the 1859 Carter specimens which are now in the Army Medical Museum at Millbank.

Fig. 9.



Sagittal section through the foot depicted on the previous page. Several large cyst like cavities are seen stuffed with black Madurella mycetomi grains. The most distally placed cyst has lost its contents revealing a smooth glistening lining. Large parts of the foot show complete destruction and replacement by accumulations of grains and fibrous tissue. Muscle bellies and cartilage nearby have remained healthy.

Fig 10.

discuss this specie in detail before going on to note briefly how the disease behaves when due to other agents.

MADURELLA MYCETOMI.

The pathogenesis of the infection has been described in the previous section so it does not require to be repeated here. In this specie the fundamental lesion can be termed the Madurella cyst and this arises in the following manner. At the site of implantation the organism forms small grains which break up as they enlarge thus forming further grains which begin to accumulate in a small space. By this time the original puncture wound which provided the route of entry has as a rule healed so the process goes on beneath an apparently undisturbed surface. It is a matter for speculation as to how the grains actually do increase, possibly by simple fission or else sporulation may occur in situ. Since in this specie no true spores have ever been observed in the parasitic stage the second explanation is unlikely. As the grains multiply they expand to form a cyst whose true fibrous capsule containing the grains is enclosed by a false capsule due to physical compression of the surrounding tissues. If such an early nodule is incised the grains immediately escape, indicating that they have been contained under pressure. Some of the black granules adhere to the shiny wall because of a slight mucinous lining. In my experience the grains do not have capillaries of the host entering them as is claimed by Champeau (1954). It would appear from his illustrations that his use of reticulum stains gave rise to artefacts which caused difficulties of interpretation since reticular tissue and fragments of hyphae appear to be similar. Champeau has further suggested that the colour and form of the grain depends upon the tissue with which it is in direct contact - a demonstrably false contention. However, though Champeau may err in some

MADURELLA MYCETOMI OF HAND

Transverse section through the lateral aspect of the hand of a Sudanese showing two typical 'cysts' due to Madurella mycetomi. The openings of two sinuses are visible on the volar aspect of the wrist.
This specimen is now in the museum of the Royal College of Surgeons in London and I am indebted to the curator for the photograph.

Fig 11

particulars his observations are valuable in that they do draw attention to the bland nature of the reaction which the parasite excites in the tissues of the host. The process which occurs is closely similar to the walling off of an inert foreign body by concentric layers of fibrous tissue. Histologically, fibroblasts of varying degrees of maturity are found infiltrated to varying extent with lymphocytes and histiocytes. Multinucleate giant cells may also be evident, perhaps seen in the process of ingesting peripheral fungal elements. Small capillaries in the area show endothelial proliferation and, in late lesions, almost complete obliteration as a result of a localised endarteritis. However, this foreign body, though apparently inert, is in fact a multiplying parasite which, for mechanical reasons rather than from its intrinsic invasive properties, will eventually rupture from its confining capsule. The grains thus released proceed to form a 'daughter' cyst in close proximity to the first and by a gradual continuation of this process further cysts develop forming many intercommunicating loculi. The growing tumour thus formed spreads where there is least resistance, the actual direction taken in any particular case depending upon the site of the original focus. In the presence of secondary infection, however, this rule no longer applies, as these micro-organisms cause suppuration and tissue destruction, breaking down tissue barriers and allowing more widespread dissemination of the fungal elements. The initial site determines not only the anatomical progression of the disease process but also the probability or otherwise of secondary infection and fistula formation.

The disease may present in a number of recognisably different forms in accordance with the foregoing principles.

1.
2.
3.
4.
5.
SUBCUTANEOUS NODULE
ASCENDING TYPE
SUPERFICIAL PLANTAR TYPE
'CLASSICAL' MADURA FOOT - DEEP PLANTAR TYPE
ISOLATED BONE FOCUS

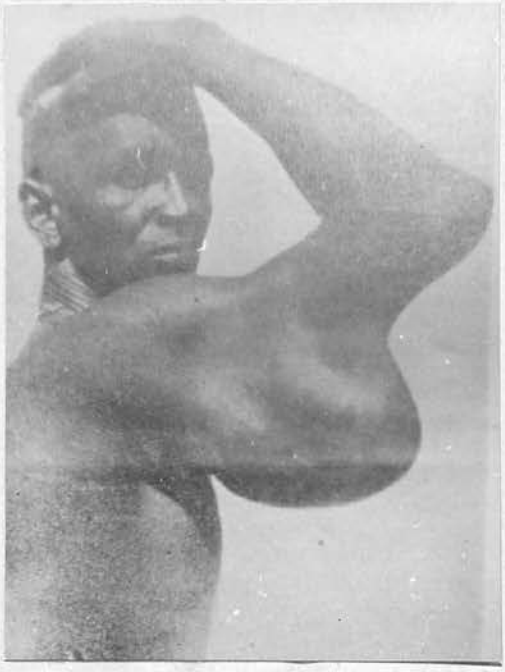
SUBCUTANEOUS NODULE.

Though this type has been rarely mentioned in the literature and has in fact been considered unusual by Vasudevan and Seshadrinathan (1929, 1930), yet it has been in my personal experience and that of other observers who have seen many more cases in Arabia, the commonest clinical manifestation. The original nidus occurs subcutaneously, superficial to the deep fascia, at a site where the skin is mobile and elastic. Thus it is common on the dorsum of the foot and around the ankle and it can also occur in many other situations. Aldridge and Kirk record a personal case from Wadi Halfa in which a small tumour the size of a marble involved the upper eyelid. They also mention two other cases involving the orbit, one affecting the lacrimal gland. The tumours may attain a great size, as is shown in the illustration overleaf where a swelling the size of a melon is present in the upper arm (Dejou and Navarrane 1953). The tumour found is frequently pedunculated, painless and on palpation is nodular and freely moveable over the underlying tissues. From its appearances and sites of predeliction it is readily mistaken on clinical examination for a lipoma, a ganglion, a sebaceous cyst or a fibrous reaction around an inert foreign body such as a thorn fragment. Unless direct trauma allows infection to enter fistualae seldom occur, the skin as a rule merely accomodates itself to the growing lesion beneath. Needless to say should grains be seen issuing from such a nodule the diagnosis will be self evident.

SUPERFICIAL PLANTAR TYPE.

The characteristic features of this type are again determined by the nature of its surroundings. In the first place thickened leathery skin covering the sole of the foot does not allow the same degree of external expansion as is found in the preceding

MYCETOMA OF ARM



Copy of an illustration appearing in an article by two French Military Surgeons Dejou & Navarrane (1953) .

This Senegalese patient presented with this massive painless tumour that had arisen over a period of years. There was no inflammation and sinuses were absent. Prior to operation a provisional diagnosis of lipoma was made. After removal the tumor was sectioned and found to consist of several loculi stuffed with black grains. This is certainly the largest subcutaneous superficial nodule on record.

Fig. 12.

variety. Furthermore, the skin of the sole is anchored in places by fibrous septa which traverse the fatty tissues and this tethering effect also limits the external bulging. Lastly, the repeated trauma of walking exerts for a time at least a further restraining influence. However, this exposed part, in an unshod individual, soon suffers some minor injury which allows pyogenic infection to enter and multiple fistula formation follows. But before this occurs the lesion can have spread widely in a horizontal plane, its deep extension being effectively restrained by the presence of the strong plantar aponeurosis. The only bones which are likely to be implicated in this type are the sesamoids of the hallux which are anatomically vulnerable. A typical case of this type is illustrated overleaf. Though this particular case involved the ball of the foot it is equally common in the pulp of the toes and in the heel.

ASCENDING TYPE.

Here the implantation has occurred in the deeper tissue planes around the muscles which, though not directly affected themselves initially, allow infiltration alongside and between them. This frequently leads to fairly extensive linear dissemination. Sooner or later, presumably due to trauma or a transient bacillaemia, infection supervenes with fistula formation. Owing to the widespread ramifications of the fungus multiple fistulae are the rule. Moore (1954) has described a case in the United States due to this organism in which extension had proceeded under the carpal tunnel connecting the forearm with the hand in a manner reminiscent of a compound palmar ganglion. Clinically pain is present its degree depending on the degree of pyogenic infection present. Destruction of tissue and reparative fibrosis may cause limitation of movement. Bone is rarely altered in this form of mycetoma. A reactionary periostitis

SUPERFICIAL MADURELLA MYCETOMI

Superficial plantar lesion due to Madurella mycetomi in an Adeni youth. A black grain can be seen issuing from the opening of a sinus. This patient was treated by excision of the affected tissues and subsequent whole thickness grafting. If the lesion had extended deep to the plantar fascia the foot would probably have had to be sacrificed. More extensive involvement of the sole would have necessitated a cross-leg or pedicle flap to close the skin defect that would have resulted from an adequate excision.

Fig. 13.

may be excited if the infective process is sufficiently close but this is more probably due to the secondary invaders. Notching of bone and subperiosteal spread with lifting up of the periosteum can occur and an X-ray showing this is in the next section.

PRIMARY BONE FOCUS.

An isolated bone focus is the least common variety of mycetoma, arising as it does from direct implantation into bone. It follows that the portion of bone usually involved is near the skin surface and generally possesses a relatively thin cortex, thus the terminal phalanges, heads of metatarsals and the os calcis are particularly vulnerable. There have also been several cases reported of large cavities occurring in the upper end of the tibia. (Grantham-Hill 1931, Kulowski and Stoval 1947, Abbott 1954). A further case, from the Aden Levy Hospital, is illustrated in the section devoted to radiology. Abbott claims that these lesions represent haematogenous embolic phenomena because their occurrence in youths and their site suggests a similar aetiology to acute osteomyelitis or a Brodie's abscess. If this view is correct one must assume that elsewhere in the body some minute primary focus is present, which has only been discovered once in the six cases reported. The case in question is the Aden one to which reference has already been made. This patient presented with a fresh lesion in the tibia two years after having a Symes' operation performed on the same side for alleged mycetoma. Another argument against Abbott's suggestion is that no lesions of an 'embolic' type have ever been discovered in viscera or in other bones. On the other hand, the upper end of the tibia in this particular age group when the tibial apophysis is not yet well developed, could readily be penetrated by a thorn, for example whilst kneeling. With so few cases available it is not meanwhile possible to decide finally between these two conflicting views of aetiology.

These primary cases in bone may go unrecognised for a long period, as pain is negligible in the early stages and the only complaint may be of a localised swelling. The cyst in the medullary cavity of the bone encounters little resistance, and is able to attain a large size without breaking up into daughter nodules. In a young subject with active osteogenesis the pressure of the grains is such that considerable expansion of the shaft of the bone may be noted. Though I have not been able to examine sections derived from cases of primary bone foci, I have studied sections of cystic lesions of bone secondary to adjacent soft tissue lesions. In the absence of pyogenic infection the concentric fibrous tissue which is such a feature of soft tissue lesions is not seen to any degree. Bone trabeculae may be in direct contact with grains which appear to be eroded without the intervention of osteoclasts. This is explicable either by pressure per se or by a humoral mechanism, but the latter theory of osteolysis is generally discredited nowadays. Grains near the periphery of such a bone focus occasionally show islets of dead bone within them. Presumably the blood supply of small portions of bone is cut off by pressure of the surrounding grains.

Further details of these bone lesions are discussed in the section on radiology.

DEEP PLANTAR TYPE.

If a primary focus is established deep to the plantar aponeurosis the prognosis is poor. Bones and joints are involved early, so that by the time the patient presents for treatment little can be offered beyond amputation. It is important to realise that the types previously described can all, in the course of time, spread into the deeper planes of the foot lead to a condition indistinguishable from one in which the deep tissues are primarily affected. The classical picture of Madura foot

is of a grossly enlarged extremity, with a convex sole, the toes unable to reach the ground and multiple fistulae discharging grains. Pain is on the whole conspicuous by its absence in Madurella mycetomi infections, though it does occur, as will be seen later, in Allescherial and Actinomycotic infections.

SECONDARY INFECTION.

Though secondary infection occurs around the ostia of sinuses and directly beneath them, it is rare to find any evidence of pyogens deep within a Madurella mycetomi lesion. This explains on the one hand the absence of pain and it also suggests a reason why cartilage and joint spaces usually remain intact in even the most extensive instances. Obliteration of joint spaces, with bony ankylosis, such as is seen particularly in Allescherial infections, does not occur with this specie.

The surprising absence of infection required some explanation and it occurred to me that perhaps an antibiotic might be manufactured by Madurella mycetomi. Accordingly I grew, on nutrient and blood agar, strains of Madurella mycetomi and Phialaphora jeanselmei for two days until the colonies had become established. The following common pyogens were then plated in a radial fashion to the edge of each colony: Streptococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa and Aerobacter aerogenes. After three days no zones of inhibition were noted. This experiment does not of course parallel the nutrients and other conditions obtaining in vivo, but nevertheless it is probable that no antibiotic is formed by the parasites within the body.

SINUSES.

A sinus may arise from the original trauma which allowed the fungus to enter the tissues, but as a rule in this specie, Madurella mycetomi, the entry

This section almost passes through the mouth of a sinus in a Madurella grisea infection. The epidermis has been elevated by a polymorphic exudate which has reached the blister by a fistulous track which is outwith the plane of this particular section. The subcutaneous tissues are disorganised by dense organised scar tissue formation. which is infiltrated by lymphocytes. Small vessels show endarteritis.

Enlargement : x 17. Stained H.& E. Case of Neuhauser.

Fig 14.

wound heals completely and sinuses arise at a later stage and not necessarily at the site of entry.

At first a small hard swelling appears which may be tender on pressure. Presently this enlarges and softens in the centre with breakdown of the overlying skin, allowing the escape of grains and serous discharge. Such a sinus frequently heals with scar formation though it may subsequently break down. With the closing of one exit tract others develop so that in the classical late Madura foot there are numerous scars of old sinuses alternating with recent actively discharging wounds.

The type of sinus developing in Nocardial and Streptomyces cases tends to be of a somewhat different character. The two accompanying photographs indicate better than any verbal description the large fleshy excrescences which may develop. There are multiple openings but from them no well defined tract can be followed with a sound. These proliferative sinuses consist basically of granulation tissue with a variable covering of scar tissue and unpigmented epithelium.

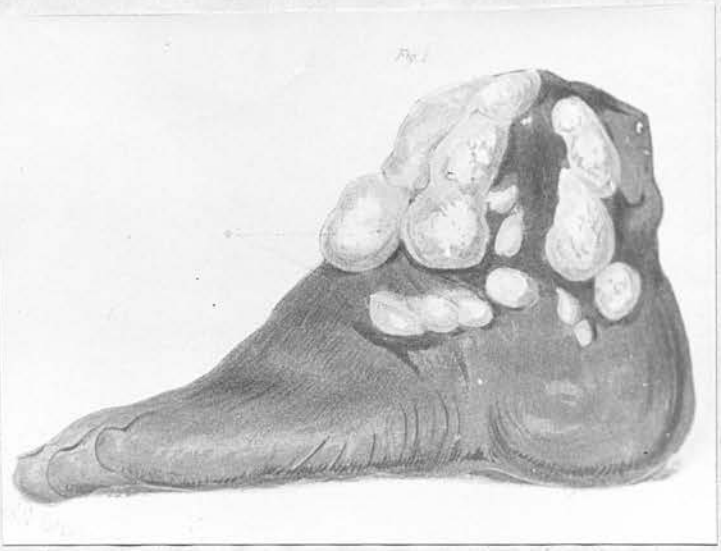
GLANDS.

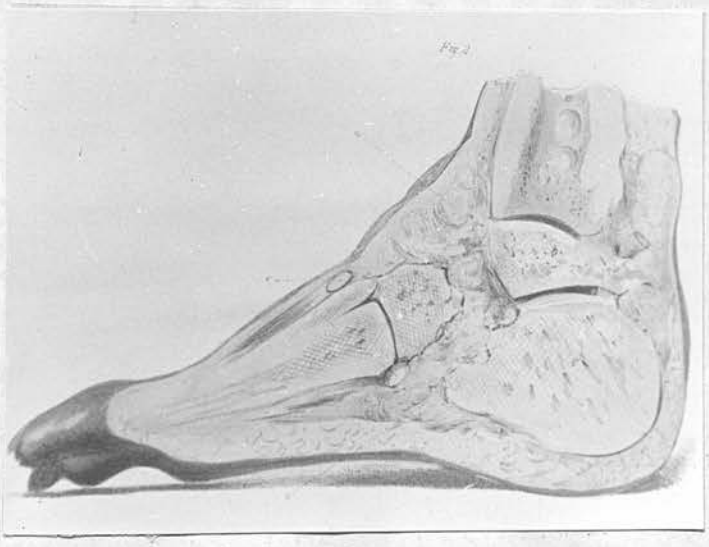
The regional lymph glands are not infrequently found to be enlarged and may be tender. This is generally due to secondary infection rather than to the presence of the fungus within the gland. Abbott reports three cases out of over 150 in which the glands contained grains of Madurella mycetomi.

In the other species glandular involvement by the fungus, though rare, is perhaps more common, having been reported by numerous authors. I include an illustration of femoral glands so affected in a Tanganyikan, and I also show microphotographs of this case and another.

MALIGNANT DEGENERATION OF SINUSES.

Chronic infective processes which give rise to





Copy of a lithograph of Carter of an 'ochroid' mycetoma. This almost certainly meant an infection from Streptomyces madurae. Be that as it may, the illustration shows very well the pale, fleshy and exuberant tissue production that frequently occurs in the Streptomyces infections.

The sagittal section of the foot demonstrates the diffuse character of the lesion which is quite different to that seen in Madurella mycetomi lesions.

Fig. 15.

NOCARDIA BRASILIENSIS

This is the foot of a nativeof the Acholi tribe of East Africa who had had mycetoma for several years. The large ulcerated areas on the dorsum of the foot shows the small openings of multiple sinuses. This fungating type of sinuses are characteristic of the Nocardial and Streptomycotic infections. This case revealed grains suggestive of Nocardia brasiliensis. Case of Mr. J.Cook,F.R.C.S.Ed.,Assistant Professor of Surgery at Makere College,Kampala,Uganda.

Fig. 16

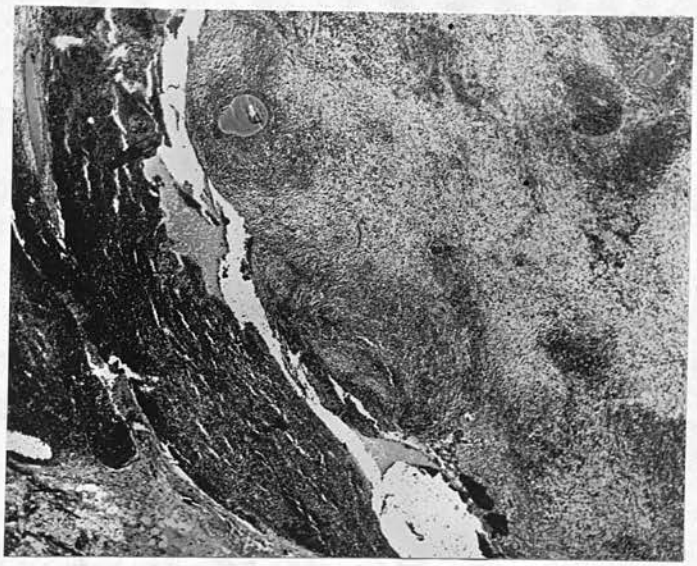
Ulcerated femoral and inguinal lymphatic glands involved metastatically from the mycetoma depicted on the previous page.

Fig. 17.

Section of a femoral lymph gland metastatically involved in the case depicted on the previous page. Apart from the increase in follicles small inflammatory foci containing grains are seen. The grains revealed features suggestive of Nocardia brasiliensis.

Enlargement : x 6. Stained by H.& E.

Fig 18.



Typical grain of Streptomyces somaliensis lying in fibrous inflammatory tissue within a lymph gland which has become metastatically involved.
Enlargement : x 17. Stained H.& E. Case of Abbott from Wad Medani in the Sudan.

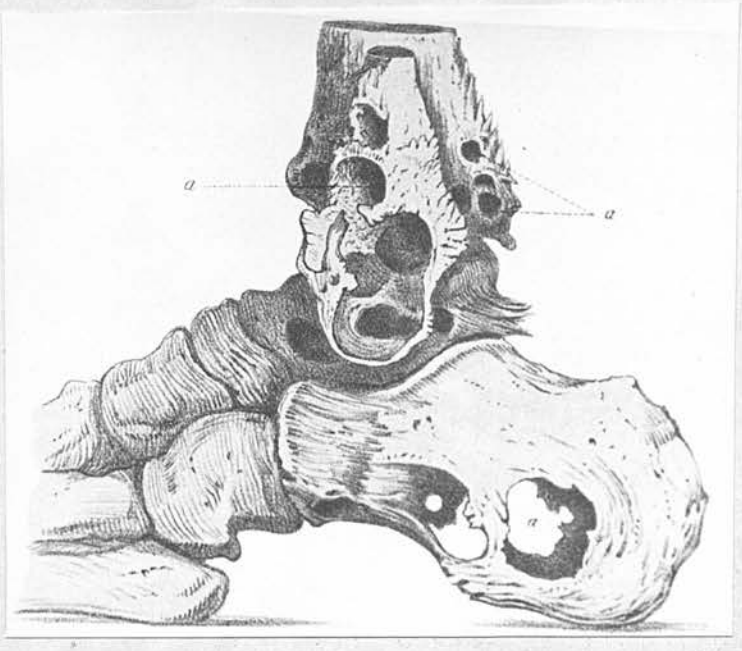
Fig 19

fistulae may on occasion develop epithelial malignant changes in a tract. This is well known to follow chronic osteomyelitis of gunshot wounds and in varicose ulcers. In a case of Actinomycosis of the anaerobic type reported by Scott (1955) an epithelioma occurred 13 years after the onset of the disease. However, as massive radiotherapy had been administered the malignancy may well have been attributable to radiation injury.

Squadron-Leader R. Pryer, F.R.C.S., saw a case of Madurella mycetomi in Aden in 1953 affecting the lower leg of an Arab. This had been present for many years though it was not possible to determine the exact duration. Multiple fistulae were present but one of these showed heaped up ulcerated edges which were clinically typical of an epithelioma. The limb was amputated and a block dissection of the regional glands was performed. The glands were normal, but the ulcer was indeed malignant. This must be a very rare occurrence as there is no other mention of such a complication in the literature.

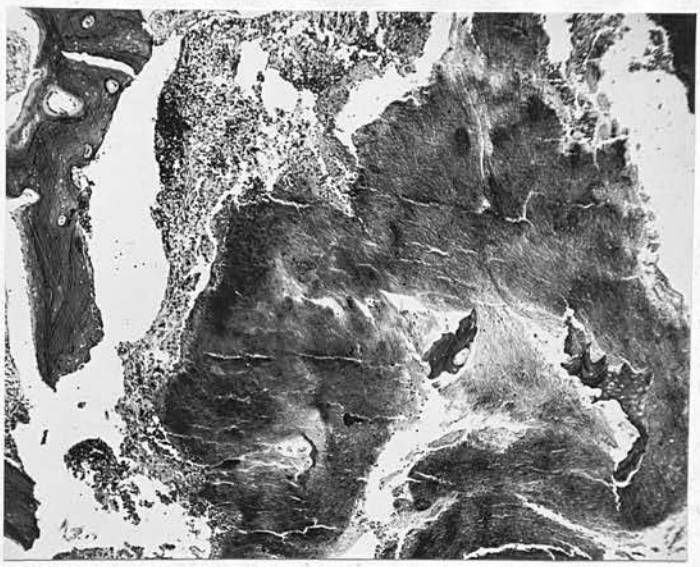
SUMMARY OF FEATURES OF MADURELLA MYCETOMI.

The changes produced by Madurella mycetomi progress slowly as the focus tends to remain localised and spread, when it does occur, takes place along tissue planes. Pyogenic infection leads to a more rapid and extensive dissemination. Pain and toxaemia are features which are conspicuous by their absence in the pure form of the infection. Lesions in bone cause well demarcated 'punched-out' defects and only following secondary infection does a more diffuse osteitis and periostitis become manifest. Articular cartilage is rarely eroded in the absence of pyogens, so that ankylosis of joints is a rarity. I have seen Madurella mycetomi grains present within a metatarso-phalangeal joint the cartilage of which was still smooth and shiny. The region lymphatic glands are very rarely involved.



Copy of a lithograph by Carter which appears in his 1874 monograph. From the accompanying history and the drawings of the microscopic features of the grains it is fairly certain that Madurella mycetomi was responsible for the infection. The typical punched out spherical bone defects are particularly well shown. The grains which were contained within these 'cysts' caused the bone defects by pressure. Periostitis is affecting the lower ends of the tibia and fibula, probably owing to the presence of secondary pyogenic infection. Particularly noteworthy is the preservation of articular cartilage.

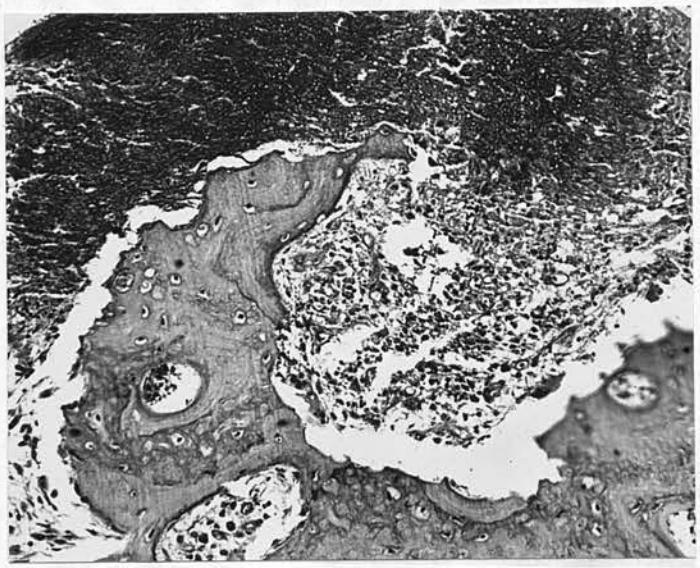
Fig. 20



Large grain of Madurella mycetomi which shows within the mycelial network fragments of bone. No osteocytes can be made out in these fragments so that they can be regarded as sequestrae. Secondary infection was present in this case but there is comparatively little reaction around this grain.

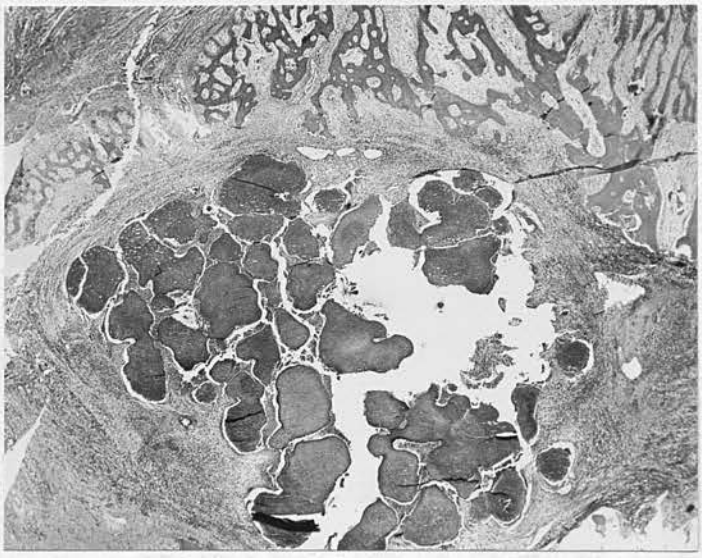
Enlargement : x 45. Stained H.& E.

Fig. 21.



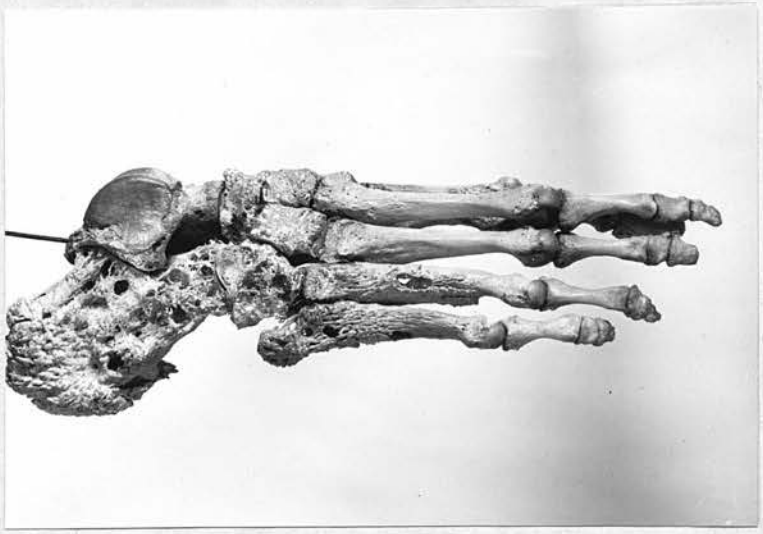
Parasitic grain of Madurella mycetomi eroding a fragment of live bone and so producing a scalloped edge.
Enlargement : x 550. Stained by a modified Schiff method.

Fig. 22.



Enlargement : x 10. Stained H.& E.
Intraosseous focus of Madurella mycetomi which had invaded bone from an adjoining soft tissue lesion. Secondary infection from sinuses may perhaps be responsible for the greater abundance of fibrous tissue in bone in this case. However it should be noted that there is no acute type of inflammatory process evident. Under the high power there is negligible osteoclastic activity and slight osteoblastic reaction. Bone may have been partly destroyed by invading fibrous tissue. Trabecular thickening is present at the edge of the focus and gives rise to the sclerotic edge noted radiographically in such cases.

Fig. 23.



Macerated specimen of the bones of the foot extensively altered by a mycetomatous process. The actual organism responsible for the condition in this case is not known. There is evidence of bone reaction to secondary sepsis and therefore I would think in view of the other changes apparent that this appearance was the end result of an infected maduromycosis.

Specimen from the Museum of the Royal College of Surgeons of England.

Fig. 24.

MADURELLA GRISEA.

Clinical accounts suggest that this specie behaves in a very similar manner to the preceding one in that pain and toxaemia are absent. Grains present deep within the lesion exist in aggregations reminiscent of those of Madurella mycetomi. However, this is not invariable, as the case of Tribedi and Muckerjee (1939) showed many grains peppered all over the cut surface though a few clumps were also visible. The radiograph of a Chilean case (Merino-Gonzalez, 1946) could well pass for that of an infected Madurella mycetomi lesion. Neuhauser's case was of the superficial plantar type and sections obtained from the deep portions showed a very bland reaction to the grains. Giant cells are frequent and an example of this is illustrated.

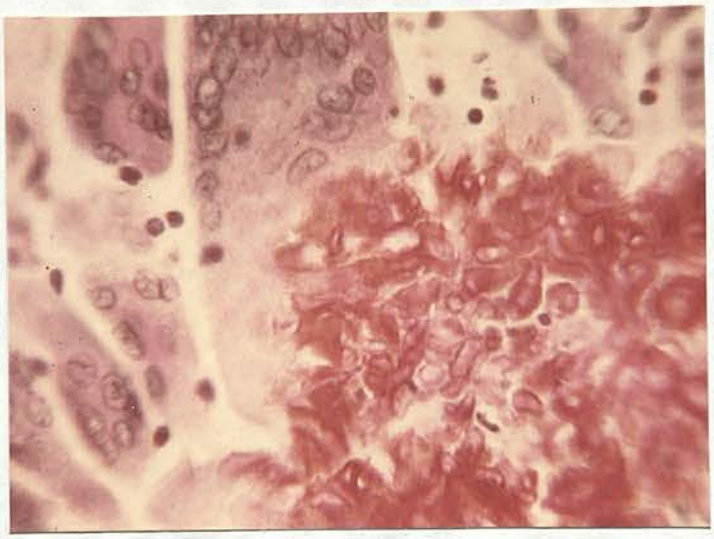
CEPHALOSPORIUM.

I have insufficient information to know what is the typical clinical picture in this type of maduro-mycosis. The descriptions, sections and photographs which I have seen suggest that this type of infection behaves in a manner intermediate between the relatively bland Madurella mycetomi and the more invasive Allescherial parasitisims.

ALLESCHERIA BOYDII.

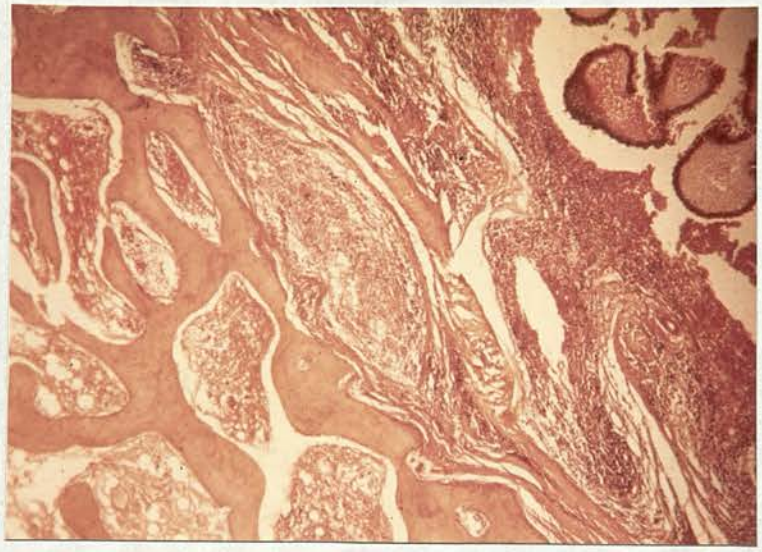
Before considering the mycetomata caused by this organism brief mention should be made of three other clinical manifestations of this fungus, as undoubtedly in the future further examples will be reported.

Case (1). A woman from the British West Indies developed meningitic symptoms following a spinal anaesthetic. Monosporium apiospermum was recovered from the sub-arachnoid space, having presumably gained entry to the theca via a contaminated lumbar puncture needle. (Aronson et al., 1953).



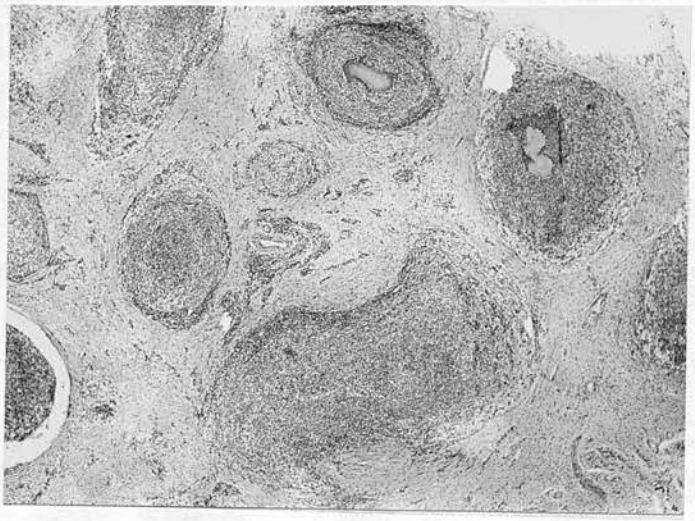
Large multinucleate giant cells are here seen around mycelial fragments of a Madurella grisea grain. This section has been stained by a periodic Schiff technique. In this particular field the grain is not typical of the specie as some of the more central hyphae are pigmented in addition to those at the periphery but in other portions of the section more typical grains were evident.

Fig 25.



Madurella grisea grains with surrounding inflammatory exudate are seen lying in relation to a tarsal bone. The periosteum which is thickened has been penetrated allowing granulation tissue to be in direct contact with cortical bone. Bone trabeculae to the left of the field are normal and are surrounded by intact fatty marrow. Case of Tribedi and Muckerjee.
(x20 aprox.)

Fig. 26



Grains of Cephalosporium sp. lying singly in the centre of small abscess systems. The individual grains are surrounded by an area of polymorph leucocytes. Nearer the edge of an abscess the exudate is more lymphocytic in character. The abscess wall is composed of granulation tissue which gradually merges into a matrix of more organised fibrous tissue which is in parts fibrinoid and degenerate. This type of reaction with dense fibrous tissue is much more frequent in the maduromycetes than the actinomycetes.
Enlargement x 20.

Fig. 27.

Case (2). A patient investigated in a hospital in Colorado on account of a chronic apical pulmonary cavity, loss of weight and cachexia was found to have a negative Mantoux reaction. Tubercle bacilli were never found in his sputum but Allescheria boydii was repeatedly recovered. (Creitz and Harris, 1955). Eventually the patient died and at the post mortem the fungus was found to be invading the lung (Creitz, 1955).

Case (3). Zaffiro, in 1938, found this organism in the blood of a patient with pyaemia. I have no further details of this report as I have only been able to obtain an abstract.

Clinically inflammation is a marked feature of infections by this fungus and pain is frequent. The sinuses which develop are similar to those of the other maduromycoses, being only slightly elevated at their ostia without any of the exuberant fleshy protruberances that characterise the Streptomycetes. Histologically the grains occur single and are surrounded by polymorphs within small micro-abscesses. The adjacent tissues are oedematous and infiltrated with lymphocytes and polymorphs. There is no attempt at encapsulation though disordered fibrous tissue is formed. Epitheliod giant cells have only been noted once (Jones and Alden, 1931). I have been given a section of a metatarsal from a case of Professor Nino. All vestige of the original cortical bone and periosteum has disappeared and has been replaced by new irregular trabeculae dispersed in a sea of fibrous scar tissue. In parts no bony elements remain and only granulation tissue and abscesses can be distinguished. No sequestra are evident and little osteoclastic activity can be discerned though there is a profusion of osteoblasts. The general impression given is of chronic osteitis which is osteoplastic and still active.

These histological bone appearances are reflected

This field is from the shaft of a metatarsal in a Monosporium apiospermum infection . The normal myeloid elements have been replaced by fibrous and granulation tissue. The increased density of this bone which was evident in the X-ray examination is seen to be due to an increase in the number of trabeculae. Many osteoblasts are visible but no osteoclastic activity could be discerned. At thetop left corner of the photograph the edge of an abscess cavity is just seen.
Enlargement : x 45. Case of Professor Flavio Nino.

Fig 28.

This low-power view reveals multiple microabscesses lying in the subcutaneous tissues. Single grains of Monosporium apiospermum are centrally placed in the abscess cavities and are surrounded by an exudate of polymorphonuclear leucocytes. The dense encircling fibrous tissue reaction which is almost constantly excited by the other maduromycetes is here absent. Enlargement : x 12. Stained H.& E.

Fig. 29.

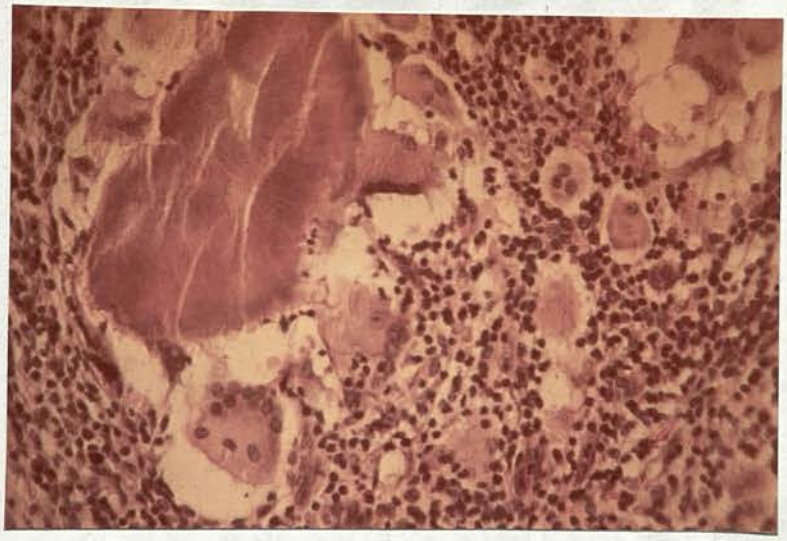
in radiographic and naked eye changes. Articular cartilage is destroyed early and fibrous ankylosis, proceeding to bony fusion, seems to be the rule in cases of any duration. Productive osteitis and periostitis with small foci of bone destruction are general. Bones at a distance from the apparent main focus may show periostitis indicating soft tissue spread. Thus, periostitis of the lower ends of the tibia and fibula has been almost a universal finding in cases involving the foot.

STREPTOMYCES PELLETIERI.

The disease as caused by this specie is the most virulent and invasive of all. The course is rapid as the lesions soon become diffuse when and if muscle planes are reached. Toxaemia does occur, perhaps from secondary invaders, and pain may be severe. The regional lymph glands frequently become involved. Several fatalities, due directly to this organism, which fortunately occurs but rarely, have been recorded. For example, a primary gluteal lesion extended into the pelvis and retroperitoneal tissues; similarly a lesion of the shoulder extended into the thorax (Madden, 1902; Dejou and Navarrane 1953). The limb roots are evidently dangerous areas in view of the invasive propensities of this fungus. Bone changes are a combination of destructive and productive lesions, with the latter predominant as dense osteitis and spiculated periostitis result.

STREPTOMYCES SOMALIENSIS.

A large number of cases of this type of mycetoma are on record, the best description available being that of Grantham-Hill (1931). All who have encountered this form of the disease will agree with him when he states that the disease shows little tendency to remain localised. Infiltration does not necessarily follow tissue planes as all the surrounding tissues may eventually become implicated in a colliquative



This section came from The Wellcome Laboratory in the Sudan. The slide was labelled '1911, Indiella somaliensis'. This was the original term given by Brumpt to Streptomyces somaliensis. The grain is totally amorphous and does not show any filaments. Giant cells and Russel bodies are present in the exudate. The latter eosinophilic structures are non specific though, at the period when this slide was made, they were regarded as indicating Botryomycosis. Giant cells are rarely seen close to a grain with filaments, where a more intense inflammation is the rule. This may perhaps indicate that a structureless grain is 'dead' since it provokes a more benign foreign body reaction.

Fig. 30

Sagittal section through the Streptomyces somaliensis mycetoma illustrated on page 182 . A ragged necrotic abscess cavity replaces a cuneiform bone. Tracts are cut in section and can be seen to extend towards the dorsum and the sole of the foot. Note the poorly limited infection which has produced changes through infiltration into the surrounding soft tissues.

necrosis. Grains do not exist in clumps or in aggregates, being dispersed throughout the lesion. Bone reacts productively though many small cavities containing granulation tissue may be formed. As secondary infection is frequent it is difficult to determine to what extent the osteitis is due to the fungus. Lymph glands are occasionally enlarged, usually from secondary infection.

Microscopically, no encapsulation of grains is noted. The totally amorphous grains may perhaps be dead and only the parasitic forms showing filaments are alive. Giant cell systems are very rare and when seen are found only in the vicinity of the amorphous granules. Russel bodies are also frequently present near these grains.

STREPTOMYCES MADURAE.

This organism shows features similar to those of Streptomyces somaliensis.

NOCARDIA.

The clinical and pathological features of mycetoma due to these organisms resemble those due to Streptomyces somaliensis.

Systemic nocardiosis is a totally different disease and will not be considered here.

SECTION VII.

THE RADIOLOGY OF MYCETOMA.

RADIOLOGY.

There are few accounts published of the radiological appearances that occur in mycetoma. There are three papers giving general descriptions of X-ray features but the emphasis is on the late stages and no distinction is made between species. (Galstaun, Tello, Castenado 1941) Numerous descriptions of the radiographic findings exist in single case reports but these are widely scattered.

Resulting from a systematic survey of the literature on mycetoma, supplemented by the loan of many X-rays and study of my own cases, I feel that certain generalisations can be made regarding the alterations that may be found in the radiograph. The classification that I propose is not intended to be rigid but rather to emphasise the forms the disease may take and to relate these forms to certain types of mycetoma.

There are three main varieties of the disease which can be distinguished radiologically,

a - the pure fungal infection.
b - the infected fungal infection.
c - the actinomycotic and Allescheria boydii infections

The specie implicated, the presence or absence of secondary infection, the site and the duration of the disease are the main variables which establish the form and appearance of the disease in any particular case.

On rare occasions the diagnosis can be suggested radiologically before it is suspected by ordinary clinical examination. On such occasions the examination is of great value. However, even in the routine clinically obvious case much information can be derived to help the surgeon. The extent of the lesion can frequently be gauged assisting the surgeon in his choice of treatment. With the development of chemotherapy for this disease serial examination will be the most valuable means of assessing progress and resolution of the infection.

SOFT TISSUES.

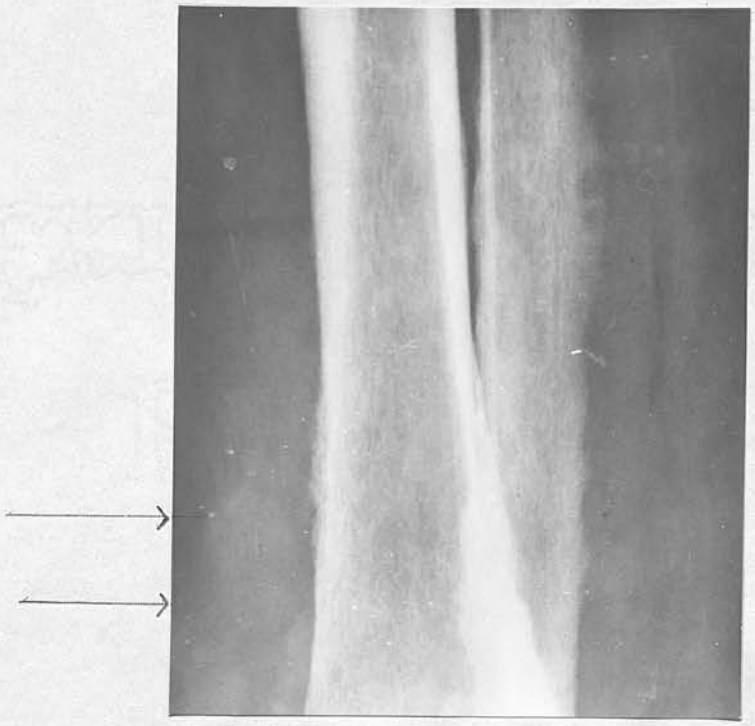
The importance of a careful examination of the soft tissue shadows in the radiograph, particularly in the early phases of the disease, cannot be overemphasised. Before bone has become involved the limits of the infective process are discernible, or may be inferred, in a suitably taken film.

Positioning and technique are of importance if an image of the required quality is to be obtained.

The region that most frequently needs to be investigated is the foot. In the study of this region the plantar aponeurosis, the muscle bellies and the tendons need to be visualised as it is upon their integrity that the form of surgery required is determined. The aponeurosis is not well delineated in the conventional oblique and lateral projections as the central beam intersects these structures at an angle. Though the ordinary lateral view may be sufficient, a modified lateral is to be preferred in order that the central beam may pass through and be parallel to the plantar fascia. The foot should be dorsiflexed and placed on its lateral side. The tube is angulated ten degrees down towards the sole, and ten degrees forwards towards the toes, and then centred just below the tuberosity of the tarsal scaphoid. Exposure factors are selected to give good soft tissue detail. When much swelling is present it is best to alter the milliampere seconds rather than the kilovoltage or penetration will be affected.

The most obvious and least important lesion seen in the film is the change in the external contour of the part. The translucent lines representing the fat which separates muscles, fascia and tendons may be displaced, blurred or perhaps obliterated.

Displacement of the lines is due typically to the lobulated tumour caused by collections of granule containing cysts, which are so typical of Madurella mycetomi lesions.



Soft tissue film to demonstrate Madurella mycetomi 'cysts'. A subperiosteal focus is seen in the shaft of the fibula. The infection in this case was ramifying in the soft tissue planes. Sinuses were present so that secondary infection is probable in this late case. The periostitis of the tibia and lower end of the fibula is evidence of secondary pyogenic invasion.

FIGURE : 31.

Blurring or obliteration occurs from the oedema of nearby infection or as a result of infiltration and scarring. This type of change is mainly seen in the actinomycotic form of the disease but can occur in the later stages of madura foot of any cause.

The extent of the lesion can be gauged more directly when the loculi of the Madurella mycetomi granuloma stand out clearly against fatty tissues or muscles by reason of the relative differences in the radiographic density of these structures. The concentric fibrosis which encapsulates collections of grains gives rise to a distinct, sharply defined, margin which is readily apparent in radiographs of the soft tissues. Implication of deep fascial planes, penetration of the plantar fascia and encroachment upon tendon sheaths may therefore be detected.

BONES.

There have been very few radiographs taken which reveal bone changes due to Phialaphora jeanselmei and Madurella grisea. Taking into account those I have seen and the histological appearances found in these species, I feel that tentatively they may be grouped together with Madurella mycetomi, though further experience may cause a modification of this view. Cephalosporium sp. seems to behave in a somewhat allied fashion but here again there is not sufficient data available to form a final decision.

Allescheria boydii is however, quite different in its behaviour clinically, pathologically and radiologically. Though the organism is a fungi imperfecta it acts in the manner of the actinomycetes.

There are many radiographs of Madurella mycetomi infections in the literature. I have examined in addition radiographs from over fifty cases. As this specie is the commonest the changes it causes will be described as representative of a pure fungal infection. The X-ray features correlate well with the histological changes.

Posterior and lateral views of great toe. The soft tissue swelling is particularly striking. The small translucency medially seen in the posterior view is due to an artefact.

FIGURE : 32.

PURE FUNGAL INFECTION.

Fungal nodules are sometimes visible in the soft tissues and bone should be examined in their vicinity. Bone is affected as a rule by the enlargement of a nodular focus near by eventually causing a localised area of absorption. A process frequently seen is the appearance of a small defect in the cortical margin with a degree of surrounding bone sclerosis. Any bone may be thus affected and Figure 32 shows the condition at work in the terminal phalanx of a great toe. At the edges of the defect there is a small area of thickening due to periosteal reaction and the periosteum has been elevated by secondary nodules growing between it and the bone. Since there is less resistance to be encountered in this plane than in penetrating the bone itself, subperiosteal spread is not infrequent. Eventually, however, the infection does penetrate bone in spite of the protective sclerosis. Within the cancellous trabeculae the fungal colonies have greater freedom of expansion than in the vicinity of cortical bone and pseudocystic lesions develop owing to absorption of bone around these intra-osseous nodules. This loss of bone results partly from osteoclasis and partly from direct pressure of the fungal colonies. In a young person the bone can actually become expanded.

The radiograph at this stage reveals a striking picture of rounded defects which may be single or multiple in the bone. There may be evidence of attempts to limit the process by sclerosis but in other areas the borders are quite clear cut and punched out. Figure 33 reveals several such cysts. Sclerosis of the fourth metacarpal is more marked, probably because slight secondary infection was clinically evident from a sinus on the dorsum of the hand. The infection has crossed the joint and has begun to involve the

Posterior and lateral view of hand of case mentioned in text. The punched out appearance of the bone defects is typical of a Madurella mycetomi lesion before there is any degree of secondary infection. The periosteal reaction and slight sclerosis indicates that sepsis has just appeared.

Fig. 33

base of the proximal phalanx. With the penetration of a joint surface the joint space is not lost as the cartilage frequently remains. The apparent immunity or enhanced resistance of articular cartilage has been mentioned in the section on pathology.

PRIMARY INFECTION OF BONE.

Fungal colonies can develop primarily in bone but this is a rare event. This form of lesion is most frequent in bones superficially situated which do not possess a thick cortex. I have seen such lesions in youths in the calcaneus and in a metatarsal head. Abbott describes a lesion of the ulna which is probably of this type. Finally there are the cystic lesions of the upper end of the tibia which are also probably of this nature.

Unfortunately the X-rays of my two cases cannot be traced in the Levy Hospital. One of the cases is, however, of great interest and in spite of the absence of actual films it merits discussion. The patient was a lad of about 7 years of age of the Maisari tribe. Most of his early life had been spent at the foothills of the Yemeni escarpment but of late he had been in Aden where his father was a senior Arab Officer. He then wore shoes and attended school. As a result of an injury to his ankle while playing he was brought to hospital and X-rayed. The film showed, as an unrelated incidental finding, a circumscribed rounded defect in the centre of the neck of his second metatarsal. The bone was expanded at this point. No soft tissue lesion was visible. The lesion was quite painless. Enquiry revealed that a deep thorn wound had occurred in that region some 6 months previously when the lad had accompanied his father on leave. A presumptive diagnosis of mycetoma was made, but unfortunately I could not persuade the father to permit exploration and resection of the lesion.

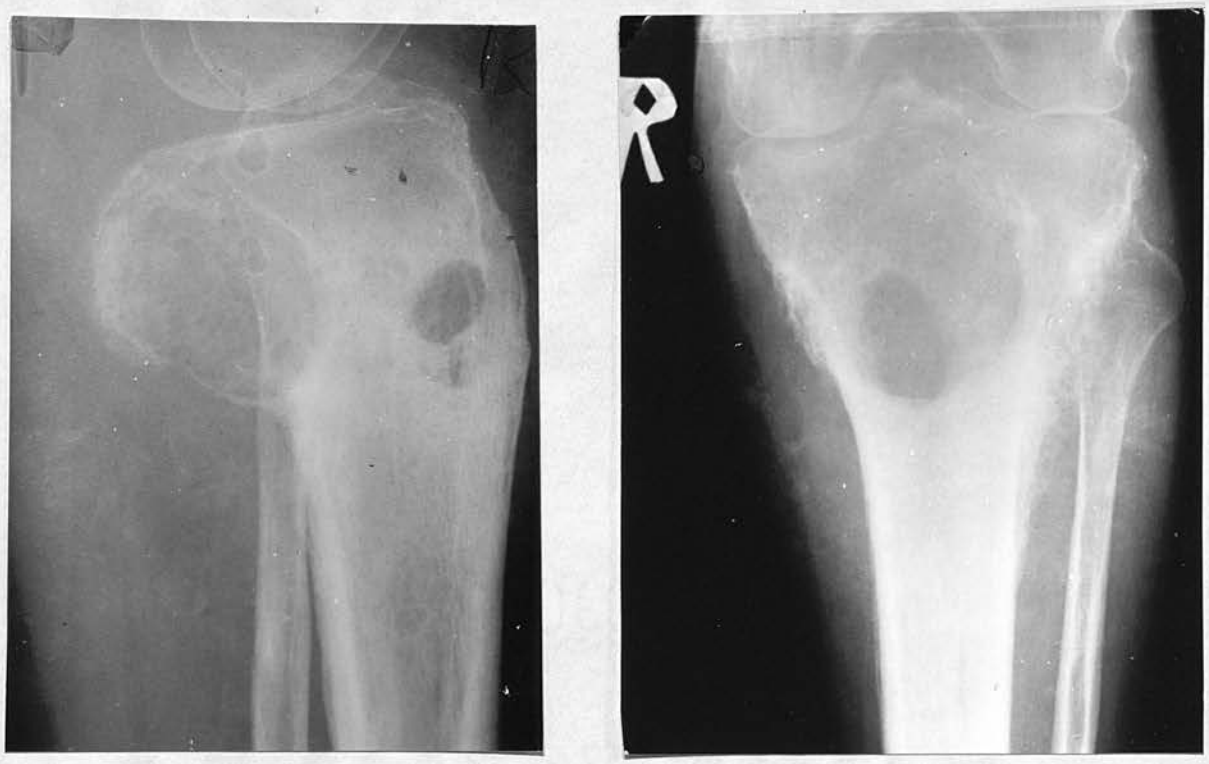
Diagramatic impression of the defect in the shaft of a metatarsal which is mentioned in text.

Recent enquiry has revealed that the foot is now swollen and discharging black grains from sinuses. (Mohammed Dean 1955, Wilkinson 1955) The diagram gives a rough indication of the X-ray findings at the time when first seen.

This type of bone change has been reported by Meyer 1930 and Sartory et al. 1930, as occurring in infection due to Hemispora stellata (Viullemin). The nature of this organism is obscure.

The best example of the pure fungal lesion in bone is that found in the upper end of the tibia in young people. Grantham Hill describes a lesion of this form. Abbott, also of the Sudan, saw three further cases. Kulowski and Stovall's case is also in this category.

The case from Aden which I illustrate was admitted to the Aden Levies Hospital in 1954. The Commanding Officer, Wing Commander Wilkinson has sent a few clinical details. The patient was an adult tribesman from the neighbourhood of Hassan, a town 50 miles inland and 150 miles measured along the coast, to the North of Aden. He gave a story of having had a swollen painful foot two years before, for which a Symes amputation had been performed at the Civil Hospital. Unfortunately no notes exist at the Civil Hospital and it is impossible to know why an amputation was performed. Presumably the original lesion was a mycetoma. On admission the Symes stump was soundly healed. A swelling waspresent at the upper end of the tibia that extended posteriorly but did not involve the joint. He said that he had had this for at least six months. A sinus was present, opening anteriorly, which exuded thin yellow pus but no granules. Microscopic examination of the exudate did not disclose any fungus Following amputation the specimen was dissected. The posterior swelling was found to be full of large black granule masses characteristic of Madurella mycetomi. The anterior lesion was separate and



Posterior and lateral views of the case referred to in the text. The anterior lesion contained no fungal elements and so can be regarded as a form of Brodie's abscess. The posterior expanding cyst was tightly packed with black grains. The periosteal reaction is probably consequent on the sepsis.

FIGURE :34.

contained pus. The anterior lesion was typical of a Brodie's abscess.

This type of bone lesion may be metastatic as Abbott contends or due to direct implantation into bone as a result of trauma. The latter route I consider to be most likely though the above case certainly lends support to a metastatic hypothesis. A haematogenous spread implies a focus of dissemination which is not present in any of the published cases. The absence of secondary lesions at other sites in mycetoma is also against vascular spread. Such 'secondaries' are surely to be expected if viable fungal elements were free in the circulation. I feel that trauma, incurred whilst kneeling, is the most likely mode of origin of this disease. All the published cases were in youngsters around the age of 11 who gave no evidence of any other lesions. Kulowski and Stovall's case was cured after local excision.

The presence of a Brodie's abscess in the case I describe is puzzling. Was this an incidental finding? The posterior expanding lesion due to the mycetomatous process was apparently bacteriologically sterile. The relation between these two lesions is a difficult one. The posterior deep-seated position of the mycetoma lesions is also difficult to reconcile with direct implantation.

Case I of Sartory et al 1930 depicts an osteolytic area in the upper tibia of a youth aged 19. The lesion was not as clear cut and situated at the epiphysis rather than metaphysis, as in the other cases. The organism was Nocardia asteroides. This would seem to be an unusual manifestation of an actinomycete infection.

INFECTED FUNGAL INFECTIONS.

Following the development of sinuses secondary infection may occur, but this is not rapid. For some obscure reason the deeper parts of the lesion

do not show changes due to infection until late. Infection can be inferred from the presence of osteosclerosis of a more diffuse type. It is not merely condensed, thin almost cortical bone that may form the periphery of a mycetoma cyst. The process is more the development of dense new bone endosteally and periosteally. Small cavities due to abscess formation may appear. Spiculated periostitis can also occur. Infection leads to the spread of the lesion so that several bones become involved. The proteolytic activity of pyogenic organisms destroys joints. The resulting fibrous ankylosis may later give rise to a degree of bony fusion. In a gross lesion it is surprising how little demineralisation of the bone occurs in spite of the inflammation. One does not see the pencilled-in margin of osteoporotic bone noted in tubercle of the tarsus which has been immobilised. The more productive fibrosis and avascularity of mycetoma may be a factor but as the theories of Leriche and Policard are now out of fashion, elaboration of this point would only mean entering into an irrelevant controversy. In the late stages this form of the disease resembles an actomycotic infection.

ACTINOMYCOTIC AND ALLESCHERIAL INFECTIONS.

These two types of organism cause similar lesions. Basically the dominant feature is a chronic productive osteomyelitis which, unlike that due to the common pyogenic cocci, involves several bones. Bones apparently not directly implicated by the fungus may also show changes of a periosteal type. This is particularly notable with Allescheria boydii which causes a productive periostitis at the lower ends of the tibia and fibula. The changes consist of a cortical sclerosis with an irregular margin, similar to that seen in the same region in response to a chronic, deep, infected varicose ulcer.

Clinically this case was a secondarily infected Madurella mycetomi lesion.By this stage it is not possibleto determine radiographicallywwhether the infection was primarily actinomycotic or a secondarily infected fungal condition. The cyst at the base of the fourth metatarsal and the intact joint spaces suggest that secondary pyogenic infection has supervened on previous pure fungal changes.

FIGURE : 35.

The bone reaction at the site of the disease is more typical. The characteristic lesion is an area of bone absorption with ill-defined edges and beyond a productive osteitis. The bone can become thickened and is often very dense. As a rule many such foci are present, not necessarily in contiguous bones The X-ray appearance is readily explained by the histological finding of numerous micro abscesses with bone destruction and a productive reaction in the trabeculae in the vicinity. The bone abscesses have rough irregular walls and many of those seen radiologically contain merely granulation tissue. The cysts are quite different in appearance and mode of formation from those occurring in Madurella mycetomi. Joint spaces are readily destroyed with resulting eventual bony fusion of the bony elements comprising the articulation. This feature is well shown in some of the cases depicted by Courtois et al.

Though irregular, thickened bones occur with disorganised trabeculation no involucrun formation or sequestra is apparent. Demineralisation of some bones may be seen.

The most valuable references on the radiological aspects of mycetoma have been indicated in the references listed at the end.

Recurrence of mycetoma following amputation. The nature of the organism is unknown. This film was sent by Dr. Kumar, radiologist at the Civil Hospital, Aden.

Fig. 36

SECTION VIII.

THE TREATMENT OF MYCETOMA.

TREATMENT OF MYCETOMA.

Though mycetoma is not a killing disease it causes considerable morbidity. In some parts of the tropics mycetoma is the most frequent cause of amputation of a limb. The effect of this on a man's life in a community near subsistence level is far more marked than in a more highly developed country. The individual can no longer till fields and yet there are no alternative sedentary tasks that he can undertake. A kneeling socket is the best prosthesis he can hope for and even this is difficult or impossible to maintain within the resources of his community.

The aim of surgery must therefore be, whenever possible, to conserve tissue and function. There are indications that chemotherapy may enable an even more conservative approach to be made. In spite of this, surgery will always be important as ablation offers a high prospect of cure.

Surgical principles will first be discussed and this will be followed by a consideration of radiotherapy, antibiotics and chemotherapeutic agents. Finally an account will be given of experimental work I have carried out on the in-vitro sensitivity of the maduromycetes to various drugs. The implication of these results and their practical bearing on treatment will be elaborated.

SURGERY.

It is fortunate that in areas where the disease is frequent patients present for treatment in the early stages as they are aware of the consequences of delay. However, in many cases when first seen there is no alternative to amputation if the disease is to be eradicated. Localised removals of toes or of the fifth toe and its metatarsal are not disabling operations. However, if the tarsal bones or the deep plantar soft tissues are implicated a

more radical operation is indicated. Mid tarsal Chopart operations are not advisable as, particularly in the late fungal or actinomycotic lesions, it is difficult to be certain that all the affected tissues have been removed. In such cases a Symes amputation is the procedure of choice. This operation produces the advantage of an end bearing stump which does not require a prosthesis.

When the tissues around the os calcis or the ankle joint itself are affected a below knee operation will have to be performed. If the kneeling stump type of 'peg-leg' is going to be supplied it is important to section bone at a lower level (5 inches below the knee) than is customary for the conventional artificial limb.

CONSERVATIVE SURGERY.

In localised lesions of the soft tissues block excision of the affected tissues is possible. This is frequently feasible in Madurella mycetomi lesions but less often so in Actinomycotic infections. The latter are often found to be ramifying more widely than had been suspected clinically.

In this type of operation several general principles should be borne in mind. The presence of sinuses indicates that sepsis is probably present. Therefore antibiotics should be given before and after operation. If no sepsis is present and it is reasonably sure that all diseased tissue has been removed primary closure of the wound can be carried out. In many instances it is wise to wait a few days before suturing the wound especially if a large dead space has been created. Skin loss consequent on surgery on the sole of the foot must be made good. If a wound is allowed to close slowly by granulation tissue and peripheral epithelialisation, deformities will occur. Skin defects can be made good by rotation or local flaps if the area to be covered is small, but when larger, direct grafting will be required.

In theory a thick pad of skin such as can be obtained from a cross-leg flap, is desirable. I have never carried out this procedure as in the two cases of this type which I have treated (figure illustrates one of these) a whole-thickness graft provided adequate cover. The eventual skin was slightly mobile and became thickened.

Localised subcutaneous nodules caused by Madurella mycetomi are readily enucleated and do not pose any surgical problem beyond that of differential diagnosis. On two occasions I had thought clinically that I was dealing with a ganglion only to find grains on sectioning the lesion. If a localised nodule is adherent dissection must be carried out with care to avoid rupture of the capsule and consequent liberation of grains into the wound.

X-ray examination is of help in determining the integrity of the plantar aponeurosis. When this is not involved the affected tissues can be removed en bloc. Similarly foci of mycetoma in muscle and other situations can be removed. If a bone is seen, at operation, to be affected locally from without it may be possible to chisel out this portion. Such a procedure is unwise in actinomycotic lesions as infection is rarely localised in bone.

Glandular enlargement from direct involvement by the fungus is rare. If the regional group of glands have formed sinuses a block dissection should be carried out.

It is of interest that in Abbott's series (1954) glands became affected post-operatively in five cases. He suggests that embolisation may have occurred.

RADIOTHERAPY.

Facilities for radiotherapy are lacking in most of the areas where mycetoma is common. There is evidence that radiation is beneficial and I feel that this form of treatment has an important role to play as an ancillary to chemotherapeutic methods.

The effectiveness of radiotherapy in treating anaerobic Actinomycosis has been confirmed by numerous reports since Bevan published six cases in 1905. On the subject of the molds and the aerobic actinomycetes few details have been published of the value of radiotherapy. Those that have appeared have had their value reduced by inadequate description of the species involved, poor follow-up and more particularly, insufficient data on the quality and quantity of radiation used. (Gavina & Auster 1937; Nino 1942; Sagher 1955; Cullen & Sharp 1951; Panja 1955). In the referred cases good results were obtained in some instances but, various antibiotics and chemotherapeutic drugs were also given.

The mode of action of radiation in this disease is certainly not a direct one on the fungal colonies. Cultures of the Wolff-Israel Actinomyces have been exposed to 50,000 r without any apparent effects. (Stokeland 1943). Repeated subcultures of irradiated strains have been carried out and these, when later compared to the parent isolate, were found to be unchanged. Thus a lethal mutant gene or other profound genetic alteration can be eliminated from consideration. It would appear that in actinomycosis the beneficial effects reside in some alteration in the host parasite relationship. The increase in tissue oxygen tension which may result from the post radiation inflammation could perhaps exert a direct detrimental effect on the anaerobic organisms. Recent work has further shown that cells and fungal spores are more susceptible to radiation in the presence of raised oxygen tension. (Stapleton & Hollaender 1952; Churchill et al 1955).

It is obvious that we are in ignorance as to how radiation can in some cases lead to improvement and perhaps cure of mycetoma as oxygen tension per se is less likely to be important in aerobic organisms. On a priori grounds the opposite would be expected,

namely spread of the disease due to the alteration by radiation of the protective limiting fibrous reaction which is such a marked feature of mycetoma. Paradoxically it is this very feature that is promising. In chemotherapy it is important to attain as high a tissue concentration of a drug as its general toxicity will permit. Fungal colonies imprisoned within fibrous abscess systems and supplied by blood vessels affected by endarteritis are placed in an excellent defensive position to withstand the onslaught of any drug. Abbott (1954) has tried to overcome these impediments by intra-arterial administration of his drugs. However, this is not a practical procedure and, in any case, the effect can only be transient. The post radiation reaction increases the vascularity of the part and possibly alters tissue permeability and thus it would allow an increase in the affected tissue concentration for the same blood level.

CHEMOTHERAPY.

The discussion of the chemotherapy of mycetoma is very difficult owing to the large number of conflicting reports that have appeared. No genuine clinical trials have been attempted but isolated successes are frequently claimed. In the large majority of instances cure is questionable owing to only a brief follow-up. Confusion is even greater in reports of in-vitro experiments. The lack of standardisation of assay techniques partly accounts for this but, of even greater importance is the natural variation existing amongst strains of a specie. Nothing can be done to overcome the latter factor so that it would seem that unless a potent drug possessing a wide spectra of activity is found, it will be necessary, in order to be rational, to determine the sensitivity of each individual specie isolated.

IODIDES.

Since a veterinary surgeon of Utrecht reported many years ago that sodium iodide would cure cervico-facial actinomycosis of cattle and humans, this halide has been regarded as a specific in fungal conditions. These compounds figure largely in the therapeutic accounts of mycetoma published in the first half of this century. The precise mode of action, if any, is not known but it has been assumed that the higher the concentration of iodides in the tissue the greater is the benefit to the patient. Oral administration was supplemented by injections. Lipiodol was particularly favoured on the continent. Indeed as recently as 1948 a case of cephalosporiosis is alleged to have been healed by injections of iodised poppy seed oil (Coutelen et al.). Dutch workers even gave iodides intravenously for eight months in a Madurella infection but the results were not spectacular (Boers et al. 1938). Local administration is probably more rational particularly in actinomycotic infections. Iodides are known to be protoplasmic poisons effective, in time, against most bacteria. Three cases of Streptomyces somaliensis infections studied in Somaliland were apparently 'cured' as the result of injection of iodides directly into the infected tissues. (Buchanan 1931). Surgery combined with iodoform packing healed a nocardial bone lesion treated in Paris (Sartory 1930).

In spite of these few good results many more cases did not benefit in any way from Iodine and indeed exacerbation was observed on a few occasions.

Palmer (1928) treated two cases of mycetoma (species not mentioned) with intravenous bismuth tartrate supplemented by the oral administration of zinc and copper citrate. The final result was alleged to be good.

STEROIDS.

Injections of pregnenolone acetate for eight months cured a mycetoma in a child caused by a Nocardia which had proved refractory to iodides, X-ray therapy, sulphonanides, penicillin and aureomycin. (Lamb et al. 1953). The authors were able to demonstrate slight sensitivity of the organism to a 1% solution of the steroid. Curtis et al. (1954) showed total inhibition of Nocardia asteroides by 0·01% diethyl-stilboestrol. Reiss (1949) demonstrated complete inhibition of a species of Nocardia by the addition of very low concentrations of diethylstilboestrol and methyltestosterone propionate to the culture medium.

A nocardia brasiliensis lesion treated in Mexico city did not respond clinically to pregnenolone acetate though diamino-diphenyl-sulphone was effective. (Gonzalez-Ochoa & Macotela 1955).

The rationale of giving steroids has not been alluded to by the above authors - the administration seems to have been largely empirical. Though the mode of action must remain conjectural some clues may lie in the metabolism of sterols by micro-organisms. Knowledge of this subject has been greatly advanced as a result of commercial production of cortisone. Many drug houses investigated the possible utilisation of micro-organisms in the build-ing up or modification of the steroid molecule.

Bacterial cells, with few exceptions, are devoid of sterols whilst this substance is synthesised by the yeasts and moulds. It is remarkable that the small section of the natural order which serves as a connecting link between the bacteria and fungi should be apparently specific in affecting the catabolism of steroids (Turfitt 1944, 1947, 1948, 1955).

Cholesterol is capable of providing the sole source of carbon to various species of Nocardia. Welsh & Heusghem (1948) have shown that strains of Streptomycetes could convert oestradiol into oestrone

when no other source of carbon was provided. The changes that have interested the manufacturing chemists have not been these simple oxidation of hydroxyl groups to keto groups but rather more fundamental changes in the sterol ring. Certain species of Actinomycetes are known to cause particular changes but few details have been released, as many of these processes are secret having been investigated by drug houses.

Under certain circumstances a sterol, or its breakdown products, may become toxic to a Nocardia. A sterol for clinical use must show no endocrine activity and must be free from possible carcinogenic effects. In conclusion it would appear that sterols may be of use in some Nocardial infections but the evidence available is not impressive.

SULPHONAMIDES.

The therapeutic efficacy of the sulphonamide group of drugs in mycetoma is disappointing except towards some Nocardial infections. An encouraging response was noted in mycetomae due to Nocardia asteroides on three occasions (Peters 1945, Calero 1947, Clark 1954). In contrast to this, other workers have not observed any significant results from the administration of these drugs in Nocardia asteroides infections (Bobbitt et al. 1955, Hager et al. 1949). Sulphadiazine was found to provide almost perfect protection in experimental Nocardia asteroides peritoneal infections of guinea pigs. (Strauss et al. 1951). Mycetoma due to Streptomyces madurae have shown no clinical benefit from sulphonamides (Duncan et al. 1939, Green et al. 1948). Diminution of secondary infection may take place in maduromycotic lesions but no direct action has been noted clinically on the fungi. Very slight in-vitro activity of sulphadiazine and sulphathiazole towards Madurella mycetomi and Allescheria boydii has been detected (Dostrovsky & Sagher 1952, Courtois et al. 1954).

I gave 'Sulphatriad' in combination with penicillin, streptomycin and aureomycin to four cases of Madurella mycetomi infections and one of Streptomyces somaliensis. Apart from the closure of some sinuses in the actinomycotic case no other significant effect was detectable after the drugs had been given in high dosage for a fortnight.

DIAMADINES.

These are a group of compounds that have been found to possess various applications in tropical medicine, mainly in the therapy of Leishmaniasis and Kala-azar. In 1945 Elson showed that propamidine possessed in - vitro antifungal activity against some human pathogens that cause superficial and deep mycoses. Subsequent clinical results in blastomycosis have been impressive.

In 1953 Fahlberg recorded complete inhibition of a Madurella sp. by 0·1 mgm/ml. of stilbamidine, propamidine, and pentamidine.

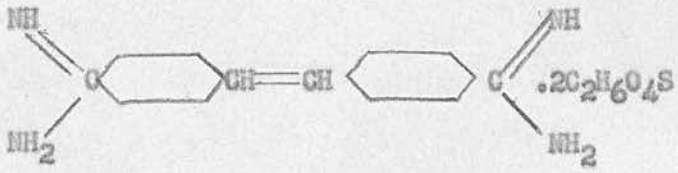
Stilbamidine, 2 - hydroxy-stylbamidine, amino-stilbamidine, propamidine and pentamidine were investigated by Curtis et al. (1954) as fungicides against, amongst other fungi, Monosporium apiospermum, and Nocardia asteroides. No compound was capable of being inhibitory at a concentration less than 1 mgm.%. Such a concentration could not be attained in the tissues without severe toxity.

Abbott (1954) determined the action of various other diamidines towards Madurella mycetomi. He was able to demonstrate a greater sensitivity than had hitherto been found. M.B. 938 (Diamidinodiphenylamine dihydrochloride) was particularly effective as total suppression of Madurella mycetomi occurred at 2 µ/gm per ml.

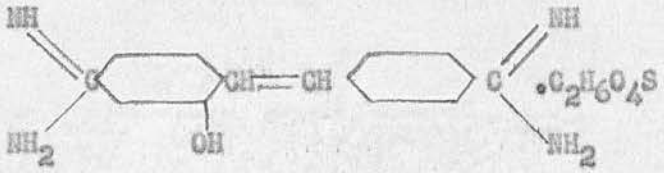
I have investigated the in-vitro sensitivity of the maduromycetes to 2 - Hydroxystilbamidine Isethionate, M.B. 938, and stilbamidine. The results are recorded in the appendix. In regard to Madurella

STILBAMIDINE

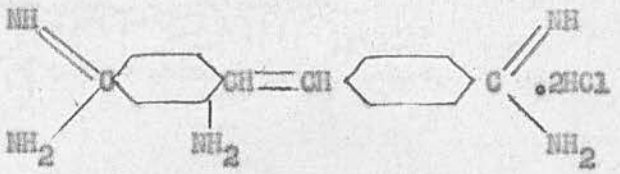
4:4' DIAMIDINOSTILBENE DIISETHIONATE



2 HYDROXYSTILBAMIDINE ISETHIONATE

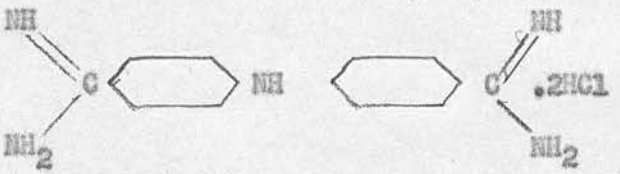


2 AMINOSTILBAMIDINE DIHYDROCHLORIDE



MAY & BAKER 938

DIAMIDINODIPHENYLAMINE DIHYDROCHLORIDE



Structural formulae of aromatic diamadines that have been mentioned in the text.

mycetomi the figures I obtained agreed qualitatively but differed quantitatively. Variations in technique no doubt accounts for this. M.B. 938 is the most potent diamadine investigated causing total inhibition of Madurella mycetomi, Monosporium apiospermum, and Phialaphora jeanselmei at a concentration of 0·2 mgm/ml. Cephalosporium falciformis was by no means as sensitive.

The toxicity of these drugs, due mainly to histamine release in the tissues, renders their clinical application dubious in the therapy of mycetoma as a sufficiently high blood level would be too toxic. Abbott gave M.B. 938 intra-arterially in treating mycetoma but though a high initial level of the drug might be secured by such means the effect could only be transient. I understand from a preliminary statement of Abbott that the clinical response was poor. Therefore though the in-vitro activity of M.B. 938 was promising it seems unlikely that it will cure mycetoma.

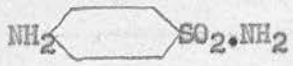
SULPHONES.

Diaminodiphenylsulphone (D.D.S.) though first synthesised in 1907 found no pharmacological application until 1937 when it was found to be effective in treating experimental strepto-coccal infections. The drug was found to be too toxic for this purpose in humans, the reason being that it was given in the same high dosage as the sulphonamides.

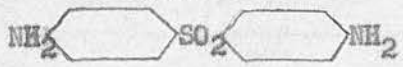
D.D.S. and a di-substituted derivative sulphetrone were later shown to possess anti-tuberculous activity but, this was soon overshadowed by the coming of Streptomycin and P.A.S. Meanwhile the parent compound and various derivatives had been shown to be of great value in the treatment of leprosy.

These results of D.D.S. on the acid fast mycobacteria responsible for leprosy and tuberculosis prompted Gonzalez-Ochoa, of Mexico City, to determine the activity of the drug against the acid fast Nocardia

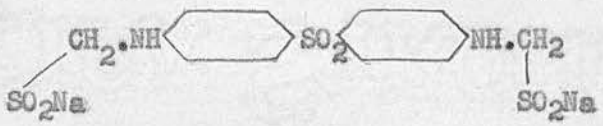
SULPHANILAMIDE



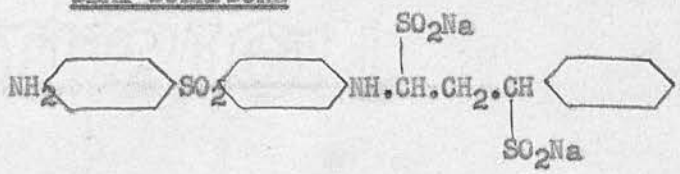
@ 4:4'DIAMINO DIPHENYL SULPHONE
(D.D.S.)



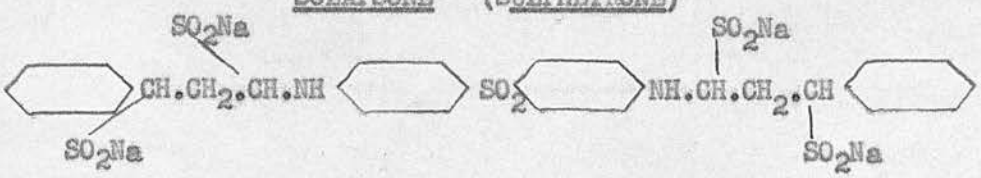
DISODIUM FORMALDEHYDE SULFOXYLATE
(SULFOXONE SODIUM U.S.P.)



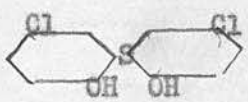
SEMI SOLAPSONE



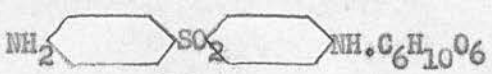
SOLAPSONE (SULPHETRONE)



2:2'DIOXY 5:5' DICHLORO DIPHENYL SULPHIDE
(NOVEX)



MONO-ANIL of 4:4'DIAMINODIPHENYLSULPHONE & GLUCURONIC ACID
(Conjugated excretion form of D.D.S.)



which cause mycetoma and Nocardiosis. In-vitro he found that D.D.S. totally inhibited Nocardia brasiliensis at 0·1 mgm/ml. and caused partial inhibition at 0·02 mgm/ml. Nocardia asteroides, Streptomyces madurae and Streptomyces pelletieri were not affected. Encouraged by these tests Ochoa started a clinical trial and has now successfully treated 24 cases of Nocardia brasiliensis infections. (Neuhauser 1955) Ochoa maintains that the drug should be given for long periods (3 years) in a similar dosage schedule to that customarily administered in leprosy. A case of Madurella grisea mycetoma treated with D.D.S. by Neuhauser has given a good clinical response but follow up is as yet incomplete. I understand that Abbott has, in a limited trial, not been impressed with D.D.S. in Madurella mycetomi infections. (Abbott 1956). Ochoa has noted differing clinical response with the D.D.S. supplied by various manufacturers. This suggests that an impurity might be partly responsible for the action.

I have investigated the in-vitro effect of D.D.S. and various derivatives on Madurella mycetomi, Monosporium apiospermum, Phialaphora jeanselmei and Cephalosporium falciformis. The drug was supplied by I.C.I. An aqeous 1% dispersion of 'Avlosulfon' was first utilised, but owing to the low solubility in water of D.D.S. it was found more convenient to use 'Avlosulfon' powder dissolved in acetone. Serial dilutions were made with acetone as the diluent. These were then added to glucose nutrient agar and autoclaved for fifteen minutes at 10lbs./sq. inch. pressure. The resulting hot liquid solution was poured into Petri dishes. After drying each plate was inoculated with all the strains being studied. Following incubation at 37°C for three days the plates were inspected. Any strain not growing was then reinoculated. Final readings which are tabulated in the tables in the appendix, were recorded after a total of five days incubation. In no case was the

was the second inoculation successful. Control plates of the pure medium and the medium plus acetone showed good growth. A further plate which contained para amino benzoic acid in addition to D.D.S. showed that the latter drug greatly diminished the antifungal activity of D.D.S. This would seem to relate the antifungal property of D.D.S. to the antibacterial activity of the sulphonamides. The structural formulae of the two both show a free amino group attached to a benzene ring.

The antileprotic activity has been believed to depend on the presence of free amino groups attached to benzene rings. Certain mono and di-substituted derivatives of D.D.S. have been prepared for use in leprosy. These are said to be less toxic though much more expensive. In the stomach these compounds are converted into the parent substance D.D.S. and this was thought to be the explanation of their action in leprosy. However, recent work has shown that they are active when injected and do not then undergo conversion to D.D.S. to any great extent. (Bushby & Woiwood 1955). The latter workers have also shown that D.D.S. exists in the body almost entirely as a conjugate of glycuronic acid with one amino group free. Thus the antileprotic action would seem to reside in a monosubstituted D.D.S. derivative.

Various derivatives of D.D.S. were obtained to determine whether the antifungal activity also depended on a free amino group. Unfortunately 'Semi-Solapsone', 'Solapsone' and 'Sulfoxone Sodium' are all unstable to heat and in solutions and would therefore decompose if the same technique of assessing activity as had been used with D.D.S. were used. At this point it is of interest to note that Sulfoxone Sodium U.S.P. has been successfully employed in curing a Streptomyces Madurae infection (Thompson & Vernon-Wax 1950).

The drug that I employed was 2,2' dioxy 5,5' dichlorodiphenyl sulphide (Novex) which is manufactured

by Boehringer and Soehne of Mannheim, Germany. In this drug there is no amino group at all so that if any antifungal activity were demonstrated the addition of para aminobenzoic acid to the culture medium would not be expected to have any effect. This proved to be the case as is shown in the tables in the appendix. These findings suggest that Novex either acts in an entirely different manner to D.D.S. or that both exert on antifungal action independently of labile amino groupings.

'Novex' is, in vitro, considerably more active than D.D.S. The most sensitive organisms tested proved to be Phialaphora jeanselmei and Monosporium apiospermum. These two fungi were partly inhibited at a concentration of 1 part per million. (·0001 mgm/ml.) Cephalosporium falciforme and the strains of Madurella mycetomi were inhibited at concentrations well below those attainable in the blood.

'Novex' is a white powder insoluble in water but readily soluble in acetone. The drug was originally developed for topical application in epidermophytosis. Tests on animals revealed negligable toxicity, cumulation does not occur, the drug being eliminated as a conjugate with glycuronic acid. (Similarly to D.D.S) Oral administration of ten 0·5 gram tablets a day leads to a blood level of about 7·8 mg/ml. The drug can also be given rectally. There is no intravenous preparation available though an oily suspension is suitable for injection into diseased tissues. The manufacturers suggest the following scheme of dosage. For an adult 12 - 15 tablets daily in divided doses continued for five days and followed by a rest of 4 days. Such a regime can be maintained for several weeks. To date the drug has produced striking effects not only in superficial mycoses but also on deep mycoses.

Several cases of anaerobic actinomycosis which had proved refractory to antibiotics have promptly respond-

ed to 'Novex' (Boehringer and Soehne - personal communication). One patient received 260 grams orally without evidence of toxicity. Richter & Temps (1952) have reported good results in blastomycotic meningitis.

Recently a case of Monosporium apiospermum mycetoma of the gluteal region was cured by a combination of surgery and 'Novex'. (Seeliger 1955).

This drug is certainly the most promising that has as yet appeared in the therapy of mycetoma. The action of 'Novex' on the aerobic actinomycetes has not been determined as yet, but I have arranged for this to be done by Professor Mackinnon who has several examples of each strain.

MYCOSTATIN.

This is an antibiotic manufactured by Squibb which is derived from extracts of cultures of Streptomyces noursei. Unlike other antibiotics commercially available at present, it was developed primarily as an antifungal agent. Candida albicans is highly sensitive to this drug both in vitro and in patients affected with thrush or diffuse pulmonary moniliasis. Torula histolytica, another yeast-like organism which causes a deep mycosis is also inhibited in the laboratory as well as being satisfactorily attacked in vivo.

Since no tests had previously been undertaken to determine whether any of the maduromycetes could be affected by this drug, simple serial dilution inhibition tests were performed (see Appendix). The two control organisms, C. albicans and T. histolytica, were inhibited at a concentration which agreed with the reports of other workers (Squibb - unpublished data, 1955). However, none of the fungi imperfecti were affected at a concentration that could be attained in the tissues. According to the manufacturers, Mycostatin does not exert any fungicidal action on any of the Nocardia or Streptomycetes.

ANTIBIOTICS.

Antibiotics have proved to be disappointing in the therapy of mycetoma. If secondary infection is present this may be limited or cured with some diminution in pain and swelling but no specific action occurs against the primary aetiologic agent. These drugs have a definite role to play in reducing the inflammation present in the tissue planes and lymphatics prior to surgery so as to ensure healing by first intention.

Numerous workers have shown that the maduromycetes are insensitive to any of the conventional antibiotics. In vitro work of Abbott (1954) demonstrated that penicillin, streptomycin and the tetracyclines were capable of inhibiting the growth of Streptomyces somaliensis, but his subsequent clinical trials proved it to be of little use though he did not administer the drug to any given case for a long period.

Penicillin and streptomycin, in combination with the sulphonamides have frequently been effective in Nocardiosis due to N. asteroids and their employment would therefore seem to be worth while should a mycetoma occur due to this organism. Benenzon (1952) claims to have cured an Allescheria boydii mycetoma by terramycin and radiotherapy. However, the follow up was short and the criteria of cure, clinical and radiographic were not sufficiently strict.

EVALUATION OF THERAPY.

A new outlook is needed amongst patients and doctors alike as regards the treatment of this chronic disease. At present the patient is usually seen by the surgeon who sceptical of the possibilities of chemotherapy and because of shortage of beds, promptly excises the diseased tissues. In the localised forms of the disease this is admirable, as it ensures a rapid cure but when the anatomical spread is extensive so that ablation would entail actual amputation at a higher

level than a Syme's, a course of chemotherapy should certainly be given an adequate trial.

In bone and joint tuberculosis, surgeon as well as patient expects a long drawn out battle with the invading organisms and is therefore prepared, even in the Tropics, to immobilise the area affected and administer drugs for months on end. A similar therapeutic patience prevails in leprosy and classical anaerobic actinomycosis is also well known to respond to prolonged courses of drugs. Mycetoma is not infrequently caused by organisms allied to the mycobacteria and Actinomyces bovis yet, apart from the little known work of Gonzalez-Ochoa in Mexico, no worker in this field has undertaken a similar optimistic and prolonged chemotherapeutic regime in mycetoma.

By analogy with actinomycosis, drugs are probably best given in combination and assessment of the result must not be made too quickly. Loss of pain, diminution of swelling and closure of sinuses are favourable, relatively early signs. Radiographically one would expect a very long latent interval before significant alterations could occur in the bone structure. It will be difficult to distinguish improvement due to the eradication of the secondary infection from that due to cure of the primary invader but this distinction will nevertheless be valuable.

Before commencing treatment it would seem to be rational and also in the interests of other workers to determine the specie of the offending organism. This may be difficult in many places owing to the absence of cultural facilities but, as has been shown earlier in this paper, a provisional diagnosis can be reached on the basis of grain morphology.

Nocardia brasiliensis mycetoma shows a high cure rate with D.D.S. Similarly, the sulphonamides combined with penicillin or a tetracycline will probably favourably influence a Nocardia asteroides

infection. The diamadines have proved disappointing in the maduromycetes clinically, though the in vitro sensitivity of Madurella mycetomi to M.B. 938 had given promise. However, Abbott was only able to exhibit the drug intermittently in any of his cases. Seeliger has recorded a favourable outcome in a case of monosporiosis with 'Novex', and I have been able to show in vitro that this drug exhibits a potent inhibitory effect on the maduromycetes. The drug is cheap, non-toxic and can be given by mouth. It therefore merits an extended trial in all maduromycoses and actinomycoses. In addition, D.D.S. can be administered since in vitro it has some action against some of these organisms and Neuhauser has obtained a good response in a Madurella grisea lesion.

SECTION IX.

MADURELLA MYCETOMI.

MADURELLA MYCETOMI (Laveran 1902).

Synonyms.

Streptothrix mycetomi	Laveran 1902
Oospora tozeuri	Nicolle & Pinoy 1908
Madurella tozeuri	Pinoy 1912
Madurella algiris	Brault & Masselot 1912
Glenospora khartoumensis	Chalmers & Archibald 1916
Madurella ramiroi	Pijara de Silva 1919
Madurella tabarkae	Blanc & Brun 1919
Madurella americana	Gammel et al. 1926
Madurella ikedae	Gammel 1927
Madurella lackawanna	Hanan & Zurett 1938
Madurella virido brunnae	Redaelli & Ciffelli 1942

Madurella mycetomi is the commonest cause of mycetoma in the world, though numerically in some parts of the world, notably South America, it provides only a small proportion of the cases.

The cases studied in India by Rustomji and Carter in the 1850's were undoubtedly due to this organism. The grains shown on page 139, from a case of Carter, are typical of the tissue forms of this specie.

Culture of the specie was probably first carried out by Wright in 1898 from a case occurring in Massachusetts. However, though he grew the organism he did not identify or name it.

Laveran in 1902 studied material from a case of Brumpt and Bouffard occurring in Djibouti in French Somaliland. He noted that the parasitic forms in the tissue differed from Streptomyces madurae and so he proposed the label Streptothrix mycetomi for this specie.

It is purely fortuitous that the designation Madurella mycetomi Laveran 1902 should have become current, based as it was on a misconception of the nature of the organism. The grains had already been described by numerous British authors and several of these had established the difference between

Streptomyces madurae and the black grain organism. Indeed Boyce and Surveyor in 1894 had shown that a Streptothrix and a fungus respectively were to blame.

Brumpt studied three further cases from Senegal in 1905 and concluded, as Boyce and Surveyor had done nine years before, that he was not dealing with a Streptothrix but a hyphomycete. For it he created the genus Madurella with Madurella mycetomi as the type specie. The botanical diagnosis was inadequate as no cultures had been obtained.

Pinoy grew a strain in 1908 in North Africa which he considered to be a new variety, Madurella tozeuri.

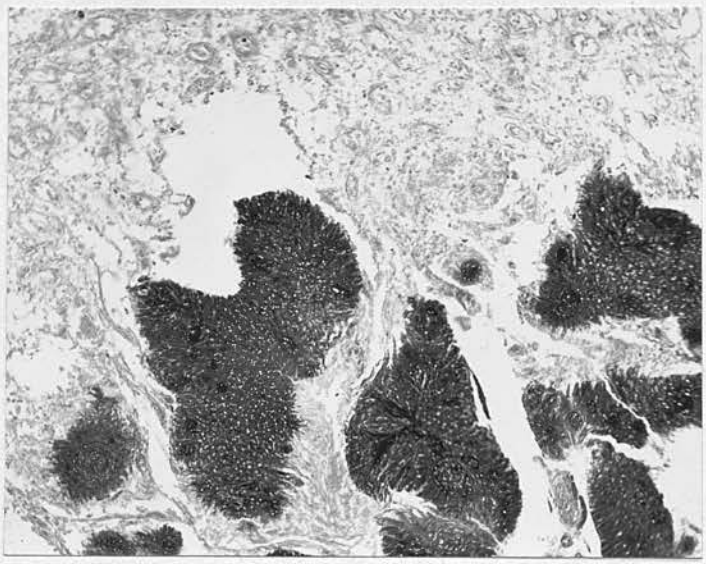
Since that period many a new culture of this specie has been dignified with a new name because of slight alleged differences between it and previous isolates. This state of affairs is reflected in the extensive synonymity that has arisen. There is little point in giving details of each strain as this would serve no useful purpose.

Mackinnon has studied over 51 strains, some were recent isolates and others had been preserved in culture collections. As a result of these studies the synonymity has been greatly reduced. (Mackinnon 1954, 1954 a).

GEOGRAPHICAL DISTRIBUTION.

The specie has been found in most parts of the world though it is infrequent in temperate climates and has not been isolated in Australia. On the map on page 32 areas where only a few isolated cases have been observed are shown separately from the 'endemic' areas.

Many of the isolated cases have not been autochthonous; e.g. Israel (Dostrovsky 1948, 1952, Sagher 1955); Malaya (Kirk 1955); The Dutch East Indies (Boers 1938) and France (Voizard & Leroy 1928). A few infections in these parts have been contracted where they were observed; e.g. Portugal (Cardoso 1939) Hungary (de Verebely 1938) and Sumatra (Boers 1938).



Grains of Madurella mycetomi from a case of Carter (circa 1859). This material was obtained from the foot shown in figures 2 and 3. In this instance chlamydospores are scanty. Mycelium is imbedded in a pigmented amorphous matrix.

Owing to the age of the preparation cellular morphology of the granulation tissue is indistinct. (Periodic stain-X 75)

Fig 37

Climate does not, as Abbott has claimed, explain the distribution of the endemic areas of the disease, as many types of climate occur within these areas.

GRAINS.

The tissue forms are always dark brown or black. The size is very variable, but the usual size is around 1 mm. in diameter. When fresh the granules are soft and can be crushed, although hardening occurs later and the grain becomes brittle.

Alkali and 'Eau de Javel' (hypochlorite) will decolourise the grains, revealing the mycelium.

In histological sections the grains are polymorphic. In ordinary haematoxylin and eosin slides it may be difficult to distinguish details because the interstitial pigment obscures the mycelium. A periodic stain enables the hyphal walls to be seen. The many varieties of grains that occur in this specie are dependent on the relative proportion of chlamydospores and mycelium present. A felted mycelium embedded in a brown matrix with peripheral vacuolated chlamydospores is the most frequent variety. Occasionally there are chlamydospores present more centrally and, more rarely still, most of the grain is composed of chlamydospores showing pigmented walls - the sclerotoid form of Mackinnon. In a single section several types of the grains may be seen which almost suggest that two species are implicated.

CULTURAL FEATURES.

Many individual descriptions of the cultural features of this organism are to be found in the literature. The most complete study is that of Mackinnon (1954) which was based on a comparitive assessment of 51 strains recovered from cases occurring in many parts of the world. Synonymity has been greatly reduced by this work. Abbott (1954) has also had extensive experience of the mycological characterisitics of this specie. I have consulted their papers freely and the summary that follows

covers the most important points.

I received ten subcultures of the specie for drug sensitivity tests. These organisms were isolated in the first instance in India, Khartoum and Wad Medani, Sudan. These subcultures revealed very markedly the polymorphism of this specie. Colonies varied in colour, surface, furrowing and degree of pigmentation. All colonies are a shade of brown but this is modified by the aerial mycelium which tends to be paler than the vegetative hyphae. A creamish aerial mycelium is present but usually only centrally so that a darker brownish rim of vegetative mycelium is uncovered. The central part of a colony is often powdery, particularly if the colony is old. All strains produce a diffusible brown pigment around the colony. Eventually the whole of the medium becomes opaque and resembles a block of chocolate. Sugars are said to facilitate the production of pigments.

Growth is most rapid at 37°. Black pin-head sclerotia appear inconstantly in the aerial or vegetative mycelium. A photomicrograph of one in section appears on page 48. The periphery is made up of fuliginous hyphae whilst the centre is composed of large unpigmented polygonal cells containing food reserves.

Microscopically the mycelium is seen to consist of branching septate hyphae 1-6 or more μ in diameter. Some hyphae bear terminal pleurogenous or intercalory chlamydospores of greater size. Though some hyphae and chlamydospores possess fuliginous walls, most contain minute brown particles. On poor media conidia may appear beneath the surface. These aleuriospores arise acropleurogenously or on short lateral branches of hyphae from which they are separated by persistent septa. (Abbott 1954) These structures had been noted by Chalmers and Archibald (1916) and Grantham Hill (1931) but had been considered specific of another specie Clenospora

khartoumensis. However, Abbott and Mackinnon were able to show that aleurospores can arise in Madurella mycetomi following culture on poor media.

Glucose, maltose and galactose were assimilated, but sucrose was not, Lactose, maltose and glucose favoured growth. Potassium nitrate, ammonium sulphate, asparagine and urea were utilised. Very slight proteolytic activity can be demonstrated. All strains hydrolysed starch.

SECTION X.

MADURELLA GRISEA.

MADURELLA GRISEA (Tribedi & Muckerjee 1939)
Mackinnon 1949.

This organism was only described as a separate specie as recently as 1949 by three South American investigators (Mackinnon, Ferrada, and Montemayor), but it had been known in the parasitic stage before and had even been cultured.

When reading Kanthack's paper of 1893 I was struck by one of the lithographs which seemed to depict the characteristic grain of M. grisea. This was of interest asat that time Mackinnon did not know of any case outside the Americas. The original specimen was traced to the Pathology Museum in St. Bartholomew's Hospital where Professor Blacklock kindly allowed me to remove some tissue for section. Histological preparations revealed undoubted grains of M. grisea. Two other probable cases from India are to be found in the literature (Chatterjee 1912 and Triberdi and Muckerjee 1939). The latter authors gave excellent illustrations of the grains and briefly noted their cultural findings. With diffidence rare in this field they remained content with describing the fungus and did not proceed to name it. They were undoubtedly the first to report culture of the species but their paper remained unknown to the South American workers until recently brought to their notice. In the interests of accuracy I have designated this specie with their name appended.

The correct generic classification is uncertain but as the species possesses some affinities to Madurella mycetomi the present name will serve though it may well be altered later.

GEOGRAPHICAL DISTRIBUTION.

The specie was isolated in Chile by Merino-Gonzalez working at Santiago. Mackinnon to whom the cultures were sent recognised that the isolate

This photograph is of the sole of afoot of a coloured woman in Chicago who was suffering from a mycetoma caused by a Madurella grisea.(case of Neuhauser) The long scar is the result of a biopsy. The raised nodules,which were soft in the centre and on occasions discharged,are typical of those seen in Madurella mycetomi lesions.

Fig 38

was of a new specie. Since then strains have been recovered from Venezuela (six times); Argentina and Brazil (twice); Paraguay (once). In the U.S.A. Neuhauser (1955) isolate the fungus from a granuloma of the sole of the foot in a patient living in Chicago.

In a review of some of the cases of mycetoma in the Armed Forces Institute of Pathology, Washington, D.C., I came across a probable case (610303) which had been labelled purely Madura Foot. (Mackinnon has confirmed my diagnosis of the species from the characteristic grains). The material was from a Filipino who had been living in Ohio. Further details were not obtainable so that it was not possible to determine where the infection was acquired.

Outside the Americas the three Indian cases already mentioned lead one to expect that M. grisea exists in other parts of the world, but there are no other definite cases, perhaps because this fungus has only been recognised as a separate entity for such a short time. Brumpt's (1906) strain of Aspergillus bouffardi from Somaliland shows grains which are indistinguishable from M. grisea. The generic designation of Aspergillus was given with little justification as cultures were not made. Brumpt claimed to have seen typical aspergillar heads in the grains, but these were not evident to Mackinnon who recently studied his material when visiting Paris. Similarly the case of Balfour (1911), from the Sudan which he called Aspergillus bouffardi is to my mind in reality due to a M. grisea infection. Glenospora Semoni isolated from an Indian soldier in France by Chalmers or Archibald may have been M. grisea (Mackinnon 1954).

Typical grain of Madurella grisea. The peripheral pigmented zone contrasts with the pale central mycelial network.

This is a microphotograph of a section from the Calcutta case of Tribedi and Mukherjee. I am unable to be certain of the staining technique employed in this instance. I presume it was a haematoxylin and eosin preparation.

(x 150 aprox.)

Fig. 39

GRAINS. - I have studied grains from five cases.

Grains are brown or black ranging from ½mm. to 2 mms. or more in diameter. When fresh the grains are readily crushed between cover slips, but on dying they soon harden.

Grains seen histological sections are absolutely characteristic. The most obvious feature is the deeply pigmented periphery which contrasts with the pale central hyphal network.

The central mycelium is apparent in ordinary haematoxylin and oesin preparations, but is seen far better when a periodic acid Schiff technique is employed. Then the septate nature of the hyphae is readily apparent. The hyphae are packed closely together, in contrast to the grains of Madurella mycetomi where the where the brown interstitial amorphous secretions separate the mycelial elements. This particular point is readily appreciated if grains of the latter specie are cleared and decolourised by alkali.

The peripheral pigmentation is due to the formation of a brownish substance throughout the cells at the edge of a grain. These are thicker than the central hyphae and show rounded expansions which probably represent chlamydospores.

Montemajor's (1953) case only showed hyphal elements in the tissues in the biopsy taken, no grains having been found. The illustration of a grain from Kanthack's case shows hyphae extending into the tissues. It would therefore appear that in this specie hyphae may extend without the grain and even rarely be present in small masses in the tissues of the patient.

The grains of this specie are not sclerotia.

Grain of Madurella grisea lying in muscle. The characteristic peripheral zone of pigmentation is well seen. This section was kindly provided by Dr. I. Neuhauser from her case. Her patient, a coloured woman in Chicago, responded dramatically to the administration of di-amino-di-phenyl sulphone. (H. & E. x75 aprox.)

Fig 40

CULTURAL FEATURES.

I have not been able to study any cultures myself. The following description is based on papers by Tribedi and Mukerjee (1939), Merino Gonzalez (1946), Mackinnon (1954, 1954a) and Neuhauser (1955).

On Sabouraud glucose agar growth is best at 30° producing heaped fast growing colonies. The vegetative mycelium is black covered partly by grey aerial mycelium which darkens with age. A brown reddish pigment diffuses into the medium. Two types of hyphae have been noted varying in size and shape but not in fuliginous characters of the cell walls. The most common hyphae are 1 - 3 μ in diameter but the other form of hyphae are 3 - 5 μ in diameter and moniliform in shape. The hyphae do not contain any brown particles as do those of Madurella mycetomi. Intercalary and terminal chlamydospores with thick walls are scanty. No conidia are seen.

The common sugars except lactose are utilised. Starch is hydrolysed. Proteolytic activity is slight and may be delayed in appearance.

Urea, asparagine, potassium nitrate and ammonium sulphate are capable of being used as sources of nitrogen.

SECTION XI.

PHIALAPHORA JEANSELMEI.

PHIALAPHORA JEANSELMEI LANGERON 1928.

SYNONYMS.

Torula jeanselmei	Langeron
Phialaphora jeanselmei	Emmons
Pulluluria jeanselmei	Lacaz

The nomenclature of this fungus is still a subject of dispute. The French mycologists assign the fungus to the genus of Torula of which the type specie was described by Persoon in 1796. (Langeron 1928, 1949 ; Catanei & Montpelier 1948). The Torula are a group of plant parasites. The correctness of the Continental view is difficult to assess as most members of the genus are known only in their parasitic stage as they have eluded all attempts to culture them. Reliance on the budding nature of the conidia and the toruloid character of some of the hyphae are the main taxonomic points in Langeron's insistance upon the use of the term Torula.

A further cause of confusion is the use of the name Torula for an entirely different group of fungi which are used for the production of beer and lager. Turpin in 1838 introduced this term to the brewing industry not realising that it had already been assigned. This error was difficult to rectify as the name had rapidly become familiar in the industry. Eventually the laws of botanical priority prevailed with the creation of the genus Torulopsis by Ciferri to designate the upstart.

Emmons (1945) prefers the label Phialaphora, a view that appears to have been accepted by many. The serial extrusion of conidia through an opening at the tip of a conidiophore and the flask shape of the latter, are two of the main arguments in support of this name. Most members of this genus,

which was created by Medlar in 1915, are saprophytes on wood. A further point in support of Emmon's thesis is that the name allies this organism to Phialaphora verrucosa, Phialaphora pedrosoi, and Phialaphora compactum - the agents of chromoblastomycosis. This disease is an ulcerating, usually superficial, chronic granuloma in which black grains are occasionally found. I do not agree with Lewis et al. (1948) who merely confuse this particular issue by apparently amending the clinical and mycological features of Symmers case to suit their own notions.

GEOGRAPHICAL DISTRIBUTION OF CASES.

The original case of Jeanselmei (1928) occurred in Paris in a woman who had lived in Martinique until three years before the apparent onset of the lesion.

The second report of the disease was from New York City (Symmers & Sporer). The disease followed three weeks after the introduction of wood splinters into a hand.

The only other case described occurred in Brazzaville, French West Africa. (Pelissier et al.)

PARASITIC GRAINS.

I have been able to study sections of Pelissier's 1948 case thanks to the kindness of Professor Catanei of Algiers. The reports of the other two cases contain many illustrations of the grains though Pelissier et al. do not feature any in their paper.

The grains are brown to black and very small. When fresh though the grains are firm they can be readily crushed. Seen with the naked eye the grains are suggestive of worm casts because of their whorled, coiled up tubular structure. Grains seen in histological sections tend therefore to appear as a series of separate curved or trefoil segments, the exact shape varying with the plane of the section. The photograph overleaf does not show this feature.

Enlargement : x 17. Stain : H.& E.

Group of grains of Phialaphora jeanselmei in a section from the case of Pelissier et al. 1948. Unfortunately autolytic changes and poor staining have resulted in a photograph of poor contrast and definition. It is evident however that no interstitial pigment is present and that the grains do not demonstrate the pigmented peripheral margin of Madurella grisea. The disposition of the grains as revealed in this planeof section is similar to that in the other recorded cases.

Fig 41

Grains are composed of large hyphal elements and chlamydospores. The cell walls are pigmented but there is no pigmented interstitial substance. Many of the chlamydospores contain a light yellow material. No true mycelial network is seen even with the use of Schiff stain. This lack of central mycelium may be the reason for the convoluted tubular make up of the granules.

The grains are different from those of Madurella grisea and therefore should be readily identifiable on section.

CULTURE.

Dr. J. Walker, of the London School of Tropical Medicine, sent me a subculture from Langeron's original case with which I carried out the drug sensitivity tests.

The following observations are based partly on this experience but more particularly on the excellent and detailed accounts of Langeron and Emmons.

Growth is slow at 37° but proceeds more rapidly at 25°.

On Sabouraud agar colonies are black with a markedly velvety, plush like surface which has a black, greyish, or greenish tinge according to the viewing conditions. This surface plush is due to multitudes of short perpendicular aerial hyphae which bear terminal conidiophores. The hyphae themselves contain a brown pigment but no diffusible pigment is formed. Growth on a poor medium results in more nodular colonies rather than those with the characteristic smooth circular dome like appearance. The colonies remain remarkably moist even after several weeks in striking contrast to the other maduromycoses. Colonies are indistinguishable macroscopically from those I have seen of Phialaphora verrucosa.

Slide cultures show dark septate hyphae 1-3 u in length many of which show a wasp-waist type of

indentation. Branching is not a marked feature of the vegetative mycelium but some hyphae have special spore bearing structures. These are termed conidiophores by Emmons and are found either as lateral hyphal protrusions or, more commonly, they are arranged in a radial manner at the tips of the hyphae. Conidiophores are flask shaped. Emmons states that conidia are extruded serially through the tip of the conidiophores but this is denied by Catanei. Though I saw elliptic hyaline conidia gathered together at the tips of conidiophores I have not been able to confirm or refute the view of Emmons. The conidia rarely remain in a cluster as they soon scatter with the drying of a binding substance. These scattered conidia in 24 hours, bud in a yeast like fashion.

SECTION XII.

ALLESCHERIA BOYDII.

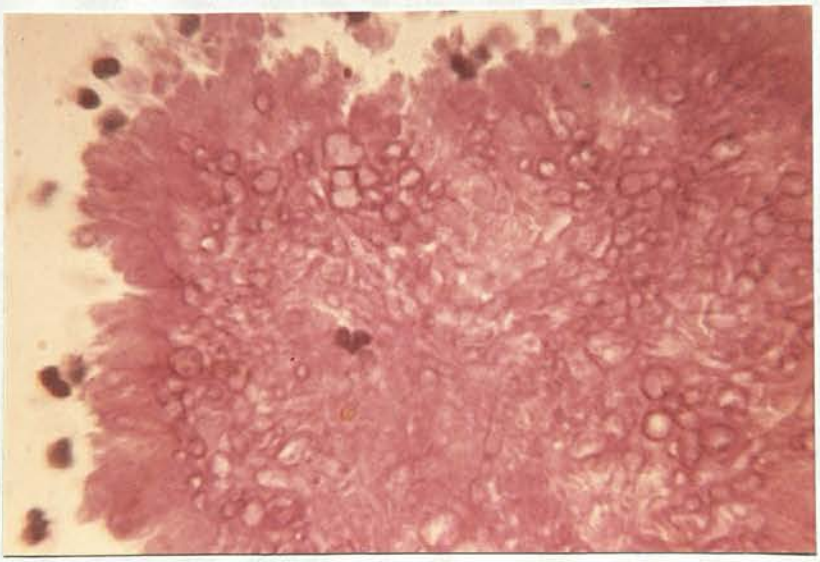
ALLESCHERIA BOYD II (Boyd &Crutchfield 1922)
MONOSPORIUM APIOSPERMUM (Saccardo 1911)

SYNONMS.

Eurotiopsis gayoni	Constantin 1896
Indiella mansoni	Brumpt 1906
Scedosporium apiospermum	Saccardo 1911
Monosporium sclerotiale	Pepere 1914
Monosporium negricans	Pepere 1914
Monosporium apiospermum var.sclerotiale	Pepere 1914
Scedosporium sp.	Magalhaes 1919
Aleurisma apiospermum	Maise 1921
Cephalosporium boydii	Shear 1922
Dendrostilbella boydii	Shear 1922
Glenospora clapiera	Catanei 1927
Glenospora boydii	Pollacci & Mannizzi 1928
Indiella americana	Delamare & Gatti 1929
Scedosporium magalhaesi	Fraes 1930
Macrosporium magalhaesi	Dodge 1935
Acromoniella lutzi	Leao & Lobo 1940
Glenospora viridobrunnae	Redaelli & Cifferi 1942
Pseudoallescheria sheari	Negroni & Fisher 1944

The above list, which has been included as a demonstration of nomenclature run riot, was derived from data provided by Cifferi & Redaeilli, Mackinnon 1948, Courtois et al. and my own review of the literature.

The first case of Monosporiosis was reported from Sardinia in 1909 by Tarozzi and the fungus was named and described by Saccardo in 1911. Boyd and Crutchfield (1921) isolated a fungus in a North American maduromycose which Shear considered to be an Allescheria. A. boydii resembles Monosporium apiospermum very closely in that it possesses hyphae and asexual conidia of the same form but differs as Allescheria boydii on culture forms sexual ascospores within perithecia.



Portion of grain of Monosporium apiospermum stained by a periodic Schiff technique. Club like chlamydospores are seen forming the periphery and further chlamydospores are evident in the more central parts of the granule. A few ramifying septate hyphae are also present in the interior of the grain.

Enlargement : x 450 aprox.

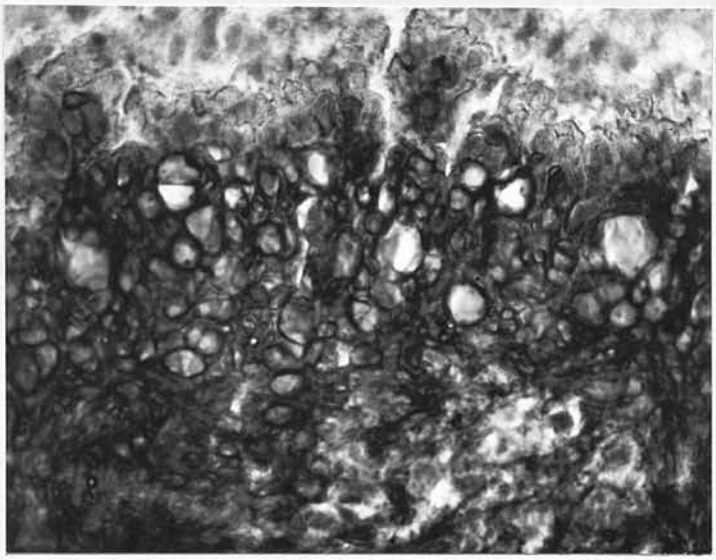
Fig 42

Emmons in 1944 compared several strains of Allescheria boydii and Monosporium apiospermum and established that the latter was the imperfect asexual stage of the former specie. The majority of isolates have been asexual on primary culture but it is possible to obtain perithecia by growing colonies of Monosporium apiospermum on a medium of low nitrogen content such 0·2% asparagine (Benham & Georg 1948, Creitz & Harris 1955.)

I have included Glenospora viridobrunnae as a synonym as Courtois et al. (1954) are of this view. From the paper I am inclined to consider this designation incorrect.

GEOGRAPHICAL DISTRIBUTION.

Most of the isolates of this specie were listed by Ajello in 1952. I will briefly summarise his findings and add to them further published and unpublished cases. The organism has been found in the soil in Tennesee, Maryland and Panama. (Ajello 1952 - 1954). All the other isolates have been from human sources though not invariably mycetoma as it has been found in septicaemia, meningitis, and a pulmonary abscess. Cases have occurred in the tropical and temperate zones of the world and on all the populated continents except Australia. Ajello mentions cases occurring in the Mediterranean littoral, the Caribbean, North and South America. He omitted from his tabulation the report of de Verebely (1938) of a mycetoma seen in Bucharest due to Indiella mansoni. The additional published cases were from the West Indies (Aronson et al. 1953), the U.S.A. (Creitz & Harris 1955), the Belgian Congo (Courtois et al. 1954), Germany (Reifferscheid & Seeliger 1955), South Africa (Lurie 1955), and Argentina (Nino 1953). I have been told of further instances in Southern India (Hancock 1955), Cuba (Castenado 1955) and Louisiana U.S.A. (Moss 1955). In addition to these there are several alleged cases on the files of the Armed Forces Institute of Pathology (U.S.A.) but I do not have details (Silliphant 1955).



High power field showing the edge of a grain of Monosporium Apiospermum. As a result of the Schiff staining the walls of the chlamydospores are clearly defined. The walls of the fungal elements at the extreme edge are not as thick or clear-cut.

Enlargement : x 600.

Case of Dr. Carlos Castenado, Havana, Cuba.

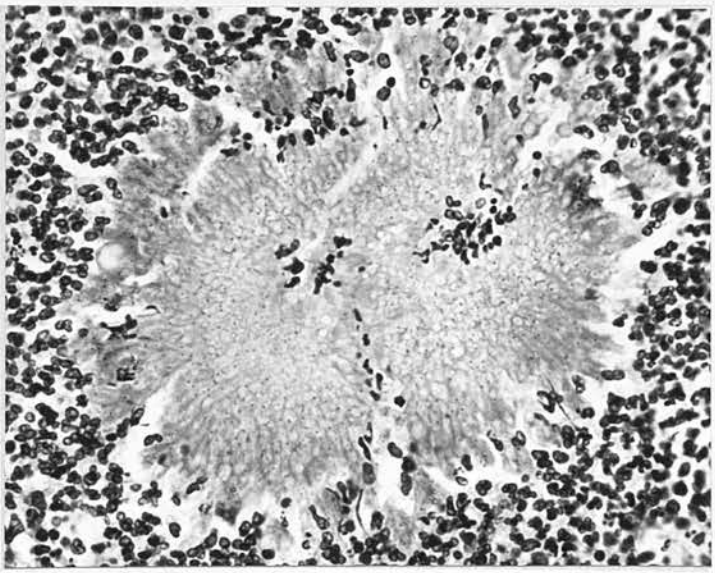
Fig 43

Amongst a miscellaneous assortment of histological preparations of Nigerian cases of mycetoma which I received from the General Hospital, Lagos there were sections from three patients that revealed typical grains of this specie. These occurred there within the last few years but no cultures had been obtained. However, as a new bacteriologist who is interested in mycology has been appointed, it may be possible to have cultural confirmation should a further instance of such a mycetoma arise.

GRAINS.

With the exception of two of the early Italian cases all the reported granules have been white or pale yellow. Their size is very variable but most are less than 1mm. When fresh, grains are soft and can be readily crushed between a coverslip and a slide. On section their shape is oval or slightly lobulated and the periphery is denticulate because of abundant large chlamydospores. The walls of the septate hyphae and the chlamydospores are best seen after a Schiff periodic staining technique has been employed. In large grains the central area may be prtly amorphous but as a rule it is composed of radially arranged mycelium and some central chlamydospores. Owing to the radial formation of the mycelium if the plane of section is not through the centre many of the hyphae are cut in transverse section rather than longitudinally. The features that I wish to emphasize are the radial mycelium, the absence of any pigment or interstitial substance and chlamydospores occurring centrally and peripherally. The hyphae are coarse 2 - 5 u in width and therefore larger than those seen in the genus Cephalosporium. The latter genus produces grains that are in many ways similar and though I consider that the points which I have emphasized differentiate the two organisms I am not certain that they alone are sufficient.

I have studied sections from six culturally proved cases and in addition there are the three probable



Grain of Monosporium apiospermum from the case of Dr. F.D.Weidman.Many chlamydospores are evident in this field, mostly in the radiating periphery. Details are somewhat obscured as the section available had been stained by Ziehl Neelsen technique.
Enlargement : x 385. Slide by courtesy of the Commandant U.S. Naval Hospital,Philadelphia,U.S.A.

Fig 44

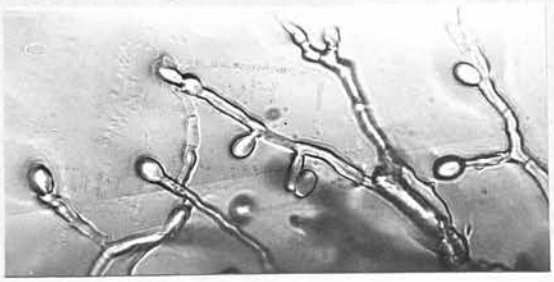
cases from Nigeria. Though there is a degree of polymorphism in the grains due to differing proportions of chlamydospores with the exception of a grain illustrated in Anderson's Textbook of Pathology all conform to the above pattern. This photomicrograph comes from a case studied in Duke University but no details are provided in the text and I have been unable to elicit any. The author states that the specie can be readily recognised in section as it is a maduromycete which is unpigmented. However, such a classification would also include members of the genus Cephalosporium and indeed the reproduction displays a grain that could well be of that genus.

CULTURAL FEATURES.

Grains grow readily after implantation in the usual media and a white colony with abundant aerial hyphae rapidly covers the plate. The surface may be 'cottony' or more velvety owing to tufts of erect conidiophores. The reverse side of the colonies show dark brown concentric rings but no pigment is diffused into the medium. This specie was the most rapidly growing of the maduromycoses that I have studied. After five days incubation at 37° C. in a nutrient agar or Sabouraud agar deep slope the whole surface of the medium would be covered by a snowy white mycelium which extended along the edges of the tube.

Sclerotia are rarely formed but have been reported and incidentally well illustrated by Gay & Bigelow (1930) and also by Fonseca & Area-Leao (1928) and Shaw & MacGregor (1935). These sclerotia show a mass of large polygonal cells invested peripherally by concentric hyphae. Such structures must not be confused with the perithecia that are seen in Allescheria boydii. These attain a large size(250u) and are filled with brown elliptical ascospores of about 4 u in diameter (Creitz & Harris 1955).

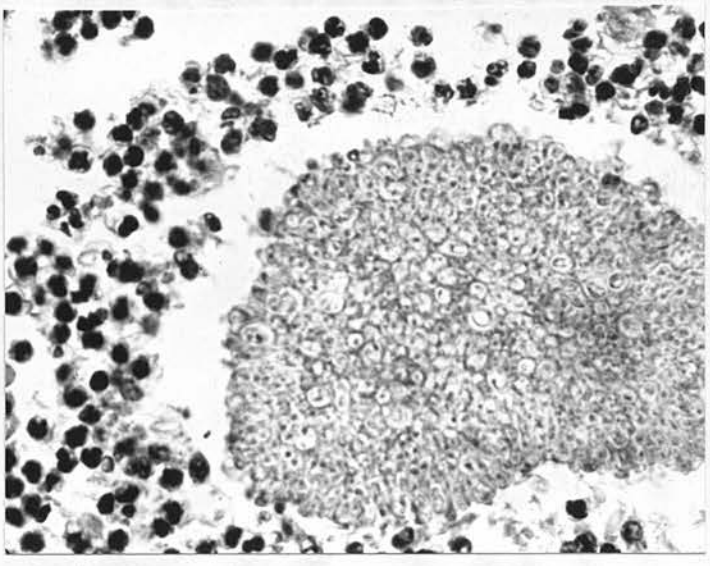
The photomicrographs of slide cultures which I



Slide culture appearance of Monosporium apiospermum showing mycelium bearing lateral and terminal conidia. Photographs kindly provided by Dr. H. Seeliger of the Hygiene-Institut of the University of Bonn.

Fig 45

reproduce reveal the characteristic appearance of the mycelium. Ovoid or pear shaped conidia are borne terminally, laterally or on short stalks in the vegetative mycelium but are usually terminal in the aerial mycelium. Hyphae of the aerial mycelium tend to stick together explaining the velvety tufted appearance noted naked eye. Some anastomosing of hyphae is noted. Conidia may show refractile particles which take Sudan III stains indicating their fatty nature (Creitz & Harris). Very complete mycological descriptions have appeared and these should be referred to further details (Dowding 1935, Carrion & Knott 1944). Animal inoculation frequently results in infection in contrast to the other maduromycoses (Gellmann & Gammel 1933; Gammel & Moritz 1933; Nino 1942; Ajello 1952).



Grain of Indiella Mansoni from a case studied by Brumpt in the Faculty of Medicine Paris. This label is considered by Mackinnon and others to be a synonym of Allescheria boydii. Few chlamydospores can be seen even at the periphery. The main mass of the grain is composed of mycelium cut in transverse section . This old slide had been stained by some form of silver impregnation.

Enlargement : 600.

Fig 46

SECTION XIII.

GENUS CEPHALOSPORIUM.

GENUS CEPHALOSPORIUM (CORDA 1839).

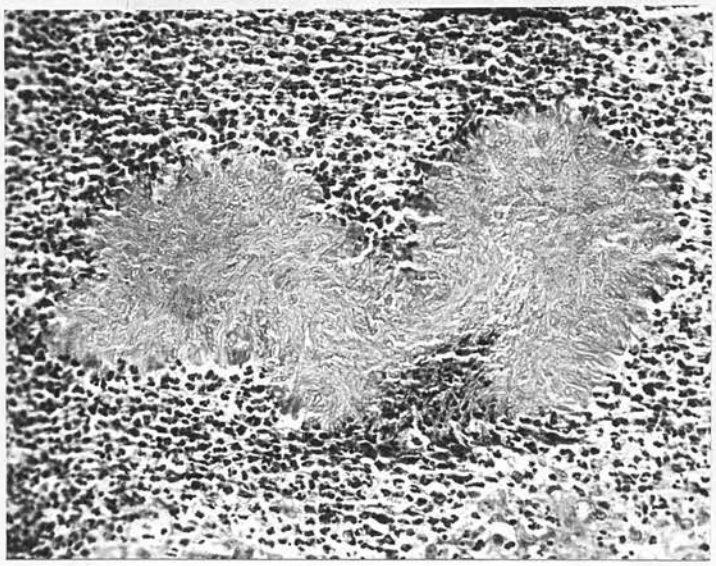
Possible Pathogenic Strains.

Cephalosporium acremonium	(Fresenius 1863)
Cephalosporium recifei	(Leas & Lobo 1934)
Cephalosporium falciformis	(Carrion 1940)
Cephalosporium granulomatis	(Wiedman & Kligman 1945)
?? Indiella reyneri	(Brumpt 1906) ??

Organisms pertaining to this genus have been found throughout the world in land, sea and in the air. Therefore it is not surprising that members of it, particularly Cephalosporium acremonium, are frequently found as contaminants in cultural work. Because of this many diverse conditions have allegedly been examples of cephalosporiosis when in fact a Cephalosporium was a chance contaminant rather than the true aetiologic agent. Most of the recorded isolates from superficial lesions and discharges are probably in this category. A possible exception is the interesting case of Douglas and Simpson (1945). This was a patient with pulmonary tuberculosis who after having an artificial pneumothorax for some time eventually developed an empyema. A Cephalosporium sp. was repeatedly recovered from the pleural fluid, but it is quite conceivable that the organism had gained entry to the pleural cavity during a refill. Various cases of sporotrichosis, tonsillitis and adenitis are much less convincing. (Miller & Morrow 1932; Coutelen et al. 1948)

The isolation of a Cephalosporium from a mycetoma should be regarded with scepticism unless certain criteria are fulfilled. In the first place, further primary cultures of material from the patient should result in the finding of an identical organism. Secondly, the grains should be of a type compatible with the diagnosis. The appearance of the grains is discussed later.

There are now a number of adequately studied cases of mycetoma in which a strain of this genus has been



Grain of a member of the genus Cephalosporium. The small whorled hyphae ,and the absence of central chlamydospores are,I consider ,typical. A coloured higher power field of this grain is shown on the next page.
Enlargement : x 250. Schiff technique stain.
Case of Dr. Lurie,Mycologist,South African Institute of Medical research.

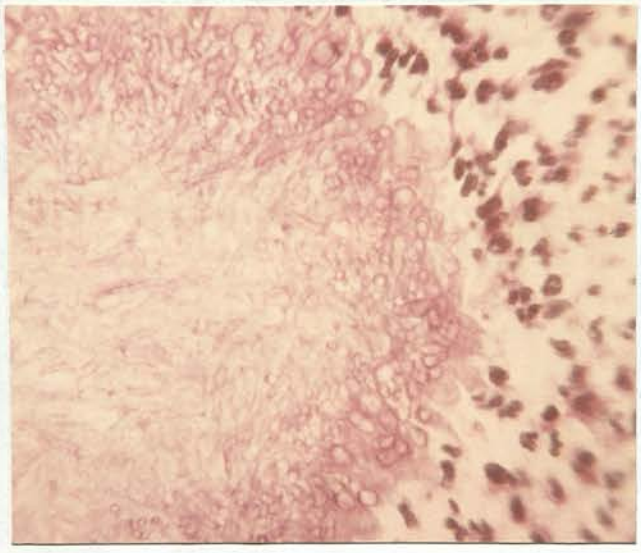
Fig 47

implicated. The number of separate species that are pathogenic is uncertain. Unfortunately mycological details of isolates are scanty and this, together with the absence of direct comparative cultural studies of isolates, makes it impossible to assess the number of different Cephalosporium that cause mycetoma. In some of the reputed instances the mycological identification did not proceed beyond the generic stage so that these cases are of little help in determining how many differing species may be involved. (Weed et al. 1949; Gonzalez-Ochoa 1944; Lurie 1955).

I believe that though there are probably at least two different pathogenic varieties of Cephalosporium little is to be gained at present in endeavouring to define and separate these as not enough is known concerning the significant features.

Cephalosporium granulomatis is either a contaminant or synonymous with Cephalosporium acremonium. Mackinnon (1951) studied subcultures of Ceph. granulomatis and identified the organism he received as Ceph. acremonium. Mackinnon thought that this invariably denotes a contaminant but I do not necessarily agree as this organism has been found to be pathogenic by Lacaz (1945) and Coutelen et al. (1948).

Mackinnon (1954) is also of the opinion that Indiella reyneri may be synonymous with a Cephalosporium. This organism was described in Paris by Brumpt (1906) purely in the parasitic form. I have noted three further instances of Indiella reyneri from Greece, one of which was confirmed by Brumpt. (Kyriasides 1930). In another of the three, cultures were obtained but the description is so poor that I could not decide whether a maduromycete or an actinomycete was in question. Certainly the inadequate photomicrographs reproduced did not show grains typical of any other species. Because of this I feel that the name Indiella reyneri is of doubtful validity as it has not been defined with sufficient



Higher power view of the grain of a Cephalosporium shown on the preceding page. The septate hyphae and chlamydospores are very well defined.

Enlargement : x 600 aprox.

Fig 48

precision. It is unlikely that it will ever be possible to know the nature of this organism.

GEOGRAPHICAL DISTRIBUTION.

Human isolates are numerous from all over the world but many of these are suspect as has been intimated above. Confining oneself to mycetoma like lesions, Cephalosporium have only been found twice outside the Americas. Once in a mycetoma in South Africa and once from a cervico-facial granuloma observed in Paris by Contelen et al. (1948).

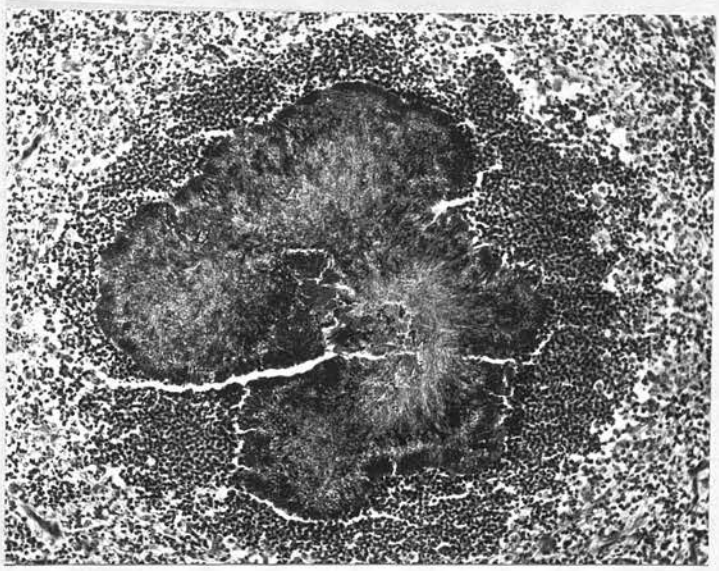
Cephalosporium recifei has been isolated in Pernambuco (Almeida and Barbosa 1940) and in Rio de Janeiro (Lacaz1955).

Cephalosporium falciformis has been reported from Puerto Rico, (Carrasquilo 1938; Carrion 1940), Bahia and Sao Paulo - the latter two towns being in Brazil. (Almeida et al. 1948).

Cephalosporium acremonium was found in a mycetoma of the thigh seen in Brazil (Lacaz 1945) and in a granuloma seen in Paris by Coutelen et al. (The latter authors mention a Japanese case which I have been unable to trace).

Cephalosporium granulomatis was noted in Philadelphia. Cephalosporium sp. mycetoma occurs in Mexico (Gonzalez-Ochoa & Ruifoba 1944), the United States (Weed et al. 1949) and South Africa (Lurie 1955).

Amongst the unidentified material I have received, two cases showed grains suggestive of this type or infection. The first was received from Rhodesia and the second from California (Armed Forces Institute of Pathology). Specimen No. 618298 in the Armed Forces Museum of Pathology in Washington, D.C., is alleged to be a Cephalosporium but I have no details of the case beyond the fact that the patient was a Filipino soldier who was treated in a military hospital in Japan. (Silliphant 1955).



Grain from a Rhodesian mycetoma which has an appearance compatible with a member of the Genus Cephalosporium. The whorled mycelium composed of thin septate hyphae and the absence of central chlamydospores are characteristic. Enlargement : x 140. Stain : H.& E.
Case of Dr. K.E.Gadd, Lusaka, Northern Rhodesia.

Fig 49

GRAINS.

In all the reported cases grains have been small and of a white or pale yellow colour. I have studied illustrations of grains from the reported cases and also sections of Lurie's South African case and Weidmann's American patient. The latter sections unfortunately did not show grains. In addition grains compatible with this specie have been noted in material emanating from Rhodesia and Japan.

Since the grains of all species show a range of variation in morphology it is difficult from the scanty data available to know that range and therefore to determine whether significant differences exist between the named pathogenic species.

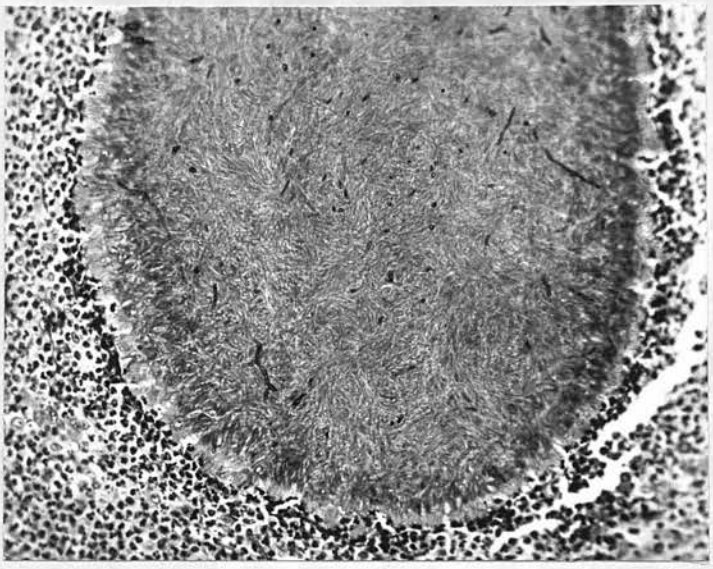
The two main features of the genus are the absence of central chlamydospores and the smaller size of the mycelium in comparison with that of Allescheria boydii.

The mycelium is composed of double walled septate hyphae arranged in whorling rather than a purely radial fashion. A Schiff stain is needed to demonstrate these details. Chlamydospores are not seen centrally, but occur at the periphery. These structures are eosinophilic. The grains tend to be ovoid or with slight lobulations. Acid fastness is not a feature of the mycelium.

I include illustrations of two grains which I provisionally place as Cephalosporium on the basis of morphology. In these cases no cultures were obtained so that it is impossible to determine the validity of this classification. It is possible that the grains are those of another undescribed specie, but nevertheless they seem to me to be compatible with those of a Cephalosporium.

CULTURAL FEATURES.

I received a subculture of Cephalosporium recifei from Professor Lacaz of Sao Paulo but unfortunately



View of a grain derived from a case of mycetoma occuring in a U.S. Army veteran in California. No cultural studies were made but the appearance of the grain is suggestive of a Cephalosporium.

Enlargement : 210. Stained by Schiff periodic technique.

Case No. 681268 in the files of The Armed Forces Institute of Pathology, Washington, D.C., U.S.A.

Fig 50

on arrival this was found to be contaminated and died out on subculture. A subculture of Cephalosporium falciformis was sent from the U.S. Public Health Mycological Reference Laboratory in Maryland, U.S.A. The gross appearance of the latter subculture appears to be identical with those of other strains described.

Growth was luxuriant at 37°c. Colonies on Sabouraud agar were creamy for the first few days but soon developed a pinkish and later a violet hue. The central part of the colony became heaped up and velvety after a week owing to the presence of an aerial mycelium. After a month colonies became brown and a brown pigment diffused throughout the medium. Secondary colonies appearing as nodules at the periphery were frequent if a culture had been disturbed or shaken.

The description of microscopic features that follows is a composite one. Mycelium is septate 1 - 1·5 μ in diameter and shows anastomoses. Perpendicular to the vegetative mycelium long conidiophores arise bearing the typical heads of Cephalosporium. The spores are arranged in clusters and are apparently held together at first by a gelatinous material which on drying sets them free. Usually five or six crescentic, falciform or banana like spores are seen in each cluster. Terminal or intercalary chlamydospores are seen at the periphery. No sexual forms of fructification such as ascospores have been noted.

Biochemical reactions are described in the literature. Glucose is usually utilised but other sugars are less so or not at all. Ammonium sulphate, potassium nitrate, histidine and peptone can supply nitrogen but urea and asparagine are not able to do so. (Contelen et al. 1948) Proteolytic activity occurs with milk.

Almeida et al. (1948) describe a positive skin reaction to a suspension of heat killed spores in their

patient.

Animal inoculation has not succeeded.

SECTION XIV.

STREPTOMYCES PELLETIERI.

STREPTOMYCES PELLETIERI (Laveran 1906).

SYNONYMS.

Micrococcus pelletieri	(Laveran 1906)
Oospora pelletieri	(Thiroux 1912)
Nocardia pelletieri	(Pinoy & Jouenne 1915)
Mycoderma griewanki	(Neveu-Lemaire 1921)
Actinomyces africanus	(Pipjer & Pullinger 1927)
Aspergillus pelletieri	(Smith 1928)
Nocardia genesii	(Froes 1931)
Actinomyces pelletieri var. hodeidae	(Ambrosioni & Merucci 1942)
Nocardia indica (Kanthack) ..	(incorrectly used by Smith 1928 and others).

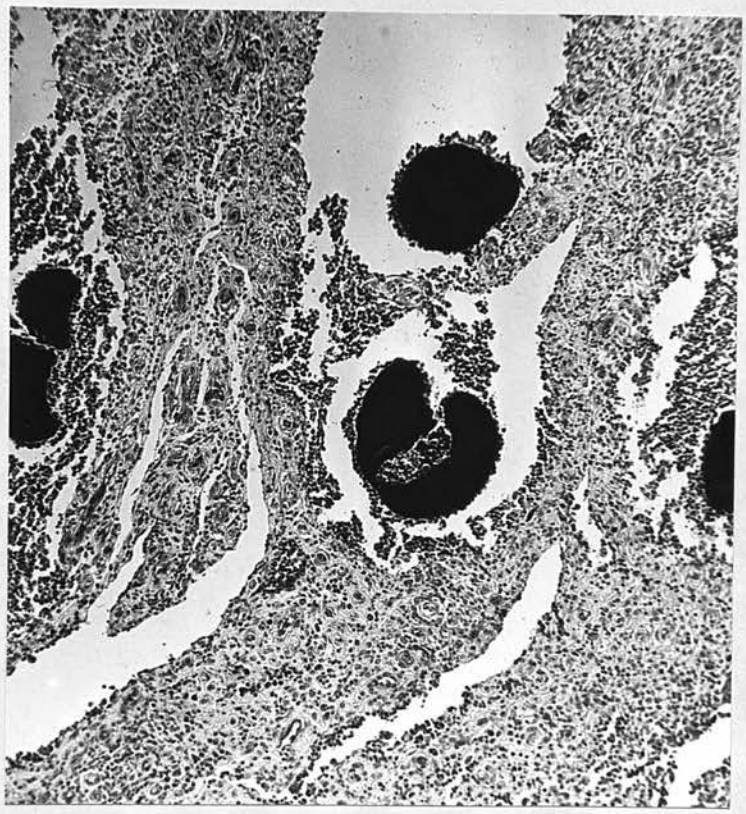
Red grain mycetoma is mentioned by Carter in his famous monograph of 1874 but he does not enter into any great details concerning an actual case though he does provide a lithograph of a foot so affected.

The first description of the grains is to be found in a very brief communication of Laveran (1906). He studied a biopsy provided by Pelletier from a native seen at St. Louis in Senegal.

Balfour (1911) described fully a case from Khartoum and published an excellent photograph of a grain but did not, it seems, obtain cultures from the grains. A fuller description of the organism, including cultural features, with details of eight cases was published by Thiroux & Pelletier (1912).

GEOGRAPHICAL DISTRIBUTION.

The specie has been reported from many parts of the world. In Northern Nigeria it is the most frequent cause of mycetoma accounting for over eighty per cent of the cases from this area. (Smith 1928, Cannon 1955 and, quoted by latter author, Elmes and Caffrey). Many cases have been observed in French West Africa, particularly Dakar and Senegal (Laveran 1906; Thiroux and Pelletier 1912; Jouenne 1915; Lecomte & Heckenroth 1916; Griewank 1919;



Streptomyces pelletieri grains imbedded in leucocytic exudate and surrounded by loose vascular granulation tissue. The grains have reproduced very darkly and do not show, at this power, any evidence of their filamentous structure. The irregular margins of the granules are due to attached lymphocytes and polymorphs.

(H.& E. x 120 aprox.)

Fig 51

Champeau 1950; Dejou & Navarre 1953).

The only British possession in this area is the small colony of Gambia that extends inland a few miles on each side of the Gambia river. This colony has similar climatic conditions to the Senegal river valley where the condition is not uncommon. For some strange reason the disease has never been seen in Gambia by any of the Medical Officers at present serving there (Jones 1955).

Streptomyces pelletieri is rare in Egypt the only cases reported from there having probably been infected in the Sudan (Madden 1902). In Northern Sudan the incidence is relatively high judging from the five cases seen by Abbott (1954) at Wad Medani during the space of two years. Balfour (1911), Grantham-Hill (1930) and Kirk (1955) have studied cases in Khartoum. I have been told, by local Medical Officers, that this form of mycetoma occurs in Somaliland and Aden. Dr. Petrie saw a case in the Yemen and a further case from the Southern part of that country was reported in great detail by Ambrosioni and Merucci (1942).

In India this form of the disease is rare, all the isolates having been obtained from the Southern half of the subcontinent. (Castellani 1919; Vasudevan et al 1928; Andleigh 1954).

Pipjer & Pullinger (1927) and des Ligneris (1928) have published accounts of red grain mycetomae in Africans of the Transvaal. In other parts of the Union no cases have been reported.

In the new world this form of the disease is uncommon. Three strains have been recovered in the Baia area of Brazil (Froes 1931; Silva 1938; Lacaz 1945). Gonzalez Ochoa and Sandoval (1951) reported a single case from Mexico.

Castenado in a letter claims to have seen two cases in Cuba in which culture was successful but I have doubts of the diagnosis of one of these as the grains were not red nor did they have the microscopic

morphology of this specie.

GRAINS.

I have examined sections from three of Abbott's Sudanese cases, a case from the Army Pathology Museum and, in addition, the description of grains to be found in published cases. The sections of Castenado and a section from Khartoum (St. Bartholomew's Museum) and one from India (Royal College of Surgeons Museum, Edinburgh), completes the list of material that was available for study.

On discharge from sinuses the grains are orange, rose or red. Some darkening to a claret colour may occur on drying. The pigment seems to be homogeneously distributed throughout the grain. The granules are small and of very variable shape. Some are rounded, oval or crescentic but most are lobulated. On section they frequently resemble the shape of a nucleus of a young polymorph granulocyte.

The grains stain well with haematoxylin and are not acid fast. The internal structure is best demonstrated after the application of a Gram stain. Then the interior is seen to be made up of numerous gram-positive filaments. In many grains these are so packed together and intertwined that few details are visible. Search usually discloses grains more loosely composed. Under the oil immersion the filaments are seen to be very fine and fragmented. Branching is not seen but small coccal bodies may be observed, particularly near the periphery.

No clubs or peripheral radial extensions occur. The edge is most often smooth though very fine denticulations are apparent under high power. Sometimes a refractile sheath is visible. Whether this is seen or not, the observer obtains the impression that a capsule of some sort is required to contain the filaments.

CULTURE.

The optimum temperature for growth is 37°.

This illustration is an attempt to demonstrate the fine Gram positive filaments contained within a grain of Streptomyces pelletieri.
Gram stain, case of Abbott from Wad Medani, Sudan.

Fig 52

Colonies develop slowly on all media. These are pinkish at first but eventually assume a rich red hue, which does not pigment the medium. Pipjer & Pullinger claim that the colonies do not show colouration if grown on media that do not contain tyrosine. This has not been confirmed. They were able to extract the pigment with ether. Jouenne (1915) notes that potash causes the colour to change to yellow.

Microscopically the mycelium is seen to be non segmented. On the branched vegetative mycelium some swellings up to 1 μ in diameter occur. No conidia were observed by Mackinnon (1955). The latter worker noted proteolytic activity but starch was not hydrolysed. Glucose is metabolised (acidification occurs but no gas) but the other sugars do not seem to be. Peptone and asparigine can serve as nitrogen sources though urea, potassium nitrate and ammonium sulphate cannot.

EXPERIMENTAL INOCULATIONS.

Mackinnon was unable to produce the disease in mice or guinea pigs. Viable filaments could be recovered four days after inoculation.

Attempts made by Smith (1928) to infect rabbits, monkeys and guinea pigs were also unavailing.

SECTION XV.

STREPTOMYCES SOMALIENSIS.

STREPTOMYCES SOMALIENSIS BRUMPT 1906.

SYNONYMS.

Indiella somaliensis	Brumpt 1906
Indielopsis somaliensis	Brumpt 1913
Nocardia somaliensis	Chalmers & Cristopherson 1916
? Discomyces somaliensis	Yazbek 1920

The original case of infection by this organism to be reported occurred in Ethiopia in 1902. Brumpt (1906) studied this case and proposed the term Indiella somaliensis for the parasite. By this designation he related the organism to the hyphomycete Indiella mansoni which is nowadays termed Allescheria boydii. Brumpt later revised his classification of the specie as he no longer considered the fungus to be a mould but rather an Actinomycete. For it he created a new genus 'Indielopsis'.

The organism was first cultured by Chalmers and Archibald (1916) working in the Sudan. They were of the opinion that a more suitable genus was that of Nocardia. Photographs contained in their article show the conidial chains in the mycelium. The latter are now regarded as one of the reasons for ascribing the specie to the Streptomyces.

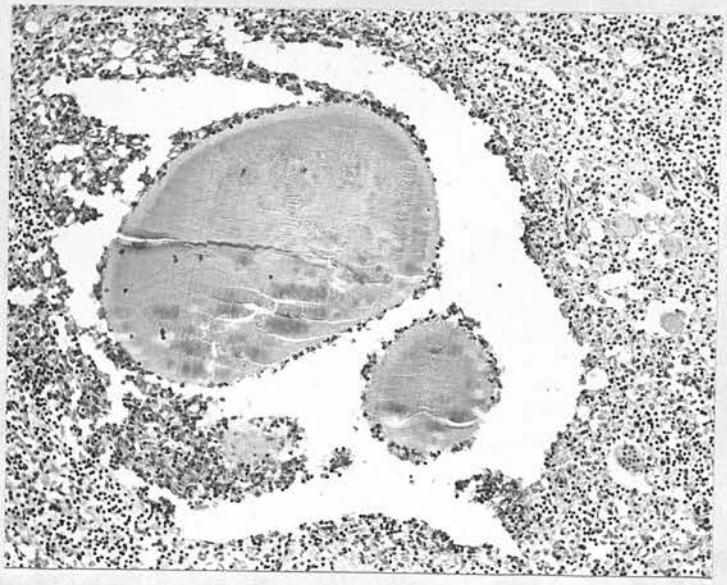
GEOGRAPHICAL DISTRIBUTION.

This specie has the most limited geographical distribution of any of the agents of mycetoma. With the exception of a doubtful case occurring in Sao Paulo, Brazil, (Yazbek 1920) no cases have been reported as arising far from the 'Horn of Africa'.

The organism has been most frequently found in the Sudan (Balfour 1911; Chalmers and Cristopherson 1916; Grantham-Hill 1931; Clarke 1953; Abbott 1954). Cases have been reported from Somaliland by Brumpt and various Italian workers. Bouffard's 1902 case was from Ethiopia. Fullerborn (1911) described a case from German East Africa. Mackinnon has details of an alleged case from Tripoli that was investigated

Mycetoma caused by Streptomyces somaliensis. A sero-purulent discharge is issuing from one of the many sinuses. The patient was a native of the Yemen.

Fig 53



This section is from the foot of the Yemeni patient which is shown on page 182. The typical structureless character of Streptomyces somaliensis grains is well shown. The pale band surrounding the grains, though it has been likened to a sheath by some authors, is not strictly so as can be seen in the next illustration which is a higher powered view of a grain. The more darkly staining basophilic patches within the grains are not unusual. No filaments are seen in these grains. Giant cells are present in the exudate. (H. &E. x110)

Fig 54

by Onarato.

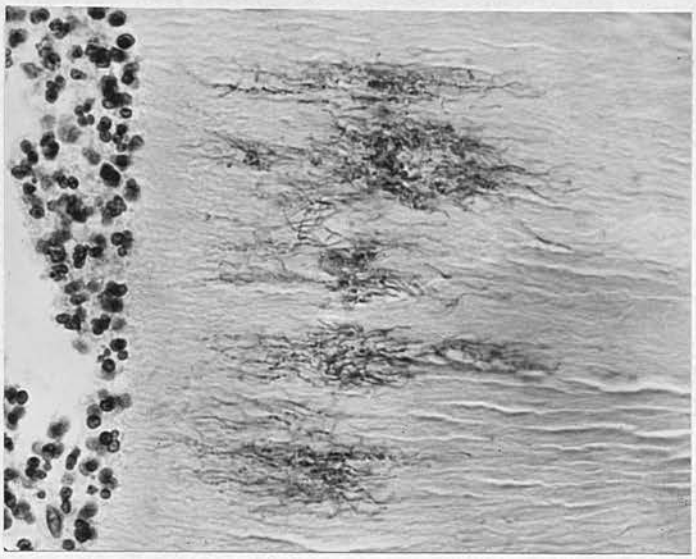
I have seen cases from the Aden Protectorate, the Yemen and Somaliland.

GRAINS.

I have studied material from my own cases, slides sent by Abbott from five cases, two cases of Archibald labelled 'Indiella somaliensis', a case from Israel sent by Altmann and, in addition, I have read descriptions and inspected photographs of cases in the literature.

The appearance of the grains in this specie is very constant. Brumpt's photographs in Plate 19 and 21 of his thesis show all the features that have been noted since. I feel that since there is so little variation in the morphology of the grains of this specie a firm diagnosis can be made from histological appearances.

The grains are small, frequently less than a millimetre in diameter. Usually they are round or ovoid though occasionally more reniform shapes may be observed. The amorphous structure of the majority of the grains, particularly in haematoxylin and oesin preparations, is probably the most noteworthy feature of the specie. With this staining technique the amorphous substance is found to be eosinophilic though a few purplish, more basophilic areas, may be present forming an internal concentric ring. Under the oil immersion lens of the microscope this ring is found to be composed of fine filaments and coccal bodies in chains. The filaments are more readily demonstrated with a Gram stain. Then they can be seen to be arranged radially close to, but never reaching the edge of the grain. These grain positive filaments are difficult to follow through their course because of the plane of section but, as a rule, some branching can be made out. This is demonstrated in the photomicrograph overleaf.



Branching filaments are seen near the periphery of a Streptomyces somaliensis grain. The continuity of the peripheral and central amorphous areas is also well demonstrated in this photograph. The peripheral band is lighter in colour and more eosinophilic but this is not readily apparent in a black and white print. The edges are well defined .

(Haematoxylin and eosin ; x550)

Fig 55

The grain is therefore composed of a structureless cement substance with a complete or partial ring of basophilic filaments. The periphery, often more eosinophilic than the central area, has a well defined edge almost suggesting the presence of a sheath. Clubs or peripheral radiating filaments are not seen. Unfortunately fixing of tissue hardens the grains so that they can be very difficult to cut. The microtome leaves parallel furrows in the amorphous substance and some grains are broken into many fragments by the process. Acid fastness is not present.

CULTURE.

Microscopically the mycelium is seen to be non segmented though occasional swellings up to 1 m in diameter have been noted by Mackinnon (1955). The filaments are Gram positive.

The cultural and biological characteristics about to be given are derived from Mackinnon's comparitive study of seven isolates.

On glucose peptone agar the colonies are creamy, but with age this darkens to a brownish hue. No diffusible pigment is formed. The gross appearance of the colonies is variable as they may be flat, furrowed or umbilicated. A lighter coloured aerial mycelium bearing conidia, typical of Streptomyces may develop but this is inconstant.

Growth proceeds best at 30° on a high energy medium with rich nitrogen content. All strains are proteolytic and amylolytic. Peptone and asparagine are assimilated but nitrates, ammonium sulphate and urea are not utilised.

Inoculations have failed to produce the disease. Mackinnon was able to recover the organisms from the peritoneum of mice fourteen days after he had experimentally introduced them into their peritoneal cavity. However, this procedure did not produce any ill effects to the animals.

SECTION XVI.

STREPTOMYCES MADURAE.

STREPTOMYCES MADURAE - VINCENT 1894.

SYNONYMS.

Oospora indica	(Kanthack 1893)
Streptothrix madurae	(Williamson 1905)
Discomyces bahiensis	(Piraja da Silva 1918)
Actinomyces brumpti	(Bordjoski & Milohevitch 1935)
Actinomyces madurae	(Duncan et al. 1939)
Nocardia madurae	(Green et al. 1948)
Actinomyces nocardia	(Carroll 1949)
Streptomyces madurae	(Gonzalez-Ochoa & Sandoval 1951)

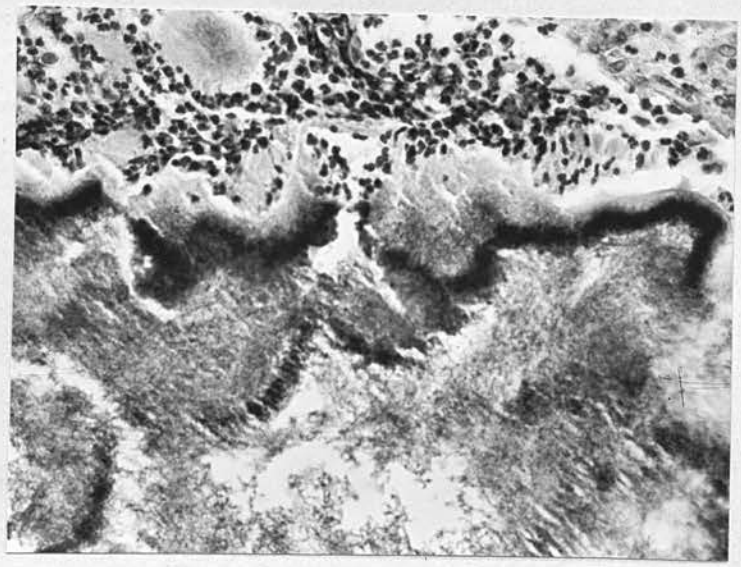
This organism, judging from the illustrations of grains, was undoubtedly that which was responsible for the 'ochroid' cases of mycetoma observed by Carter in India. Kanthack described grains from Indian cases in 1893 and named the specie Oospora indica. However, since he was under a misconception as to the nature of the fungus, which he equated with madurella mycetomi, priority of name is reserved for that given by Vincent in the following year.

Vincent cultured the organism and realised that it differed from the anaerobic actinomycetes that causes actinomycosis. Since then the organism has been found in many parts of the world under a variety of names. A small, and by no means comprehensive, list of synonyms is indicated at the top of the page. I include Discomyces bahiensis as this culture was regarded as a synonym by Yazbek 1920 and by Mackinnon 1955. The latter also considers Actinomyces brumpti to be identical with Streptomyces madurae. A. Nocardia, of Carroll, shows in the sections I have seen, identical grains to those of S. Madurae.

The presence of conidial chains on culture, noted by Vincent in 1894, is the main taxonomic basis for the placing of this specie in the genus Streptomyces.

GEOGRAPHICAL DISTRIBUTION.

This is very wide in the tropics and sub tropics. Surprisingly, in spite of the culture of many hundreds



Grain of Streptomyces madurae from the case of Altmann studied in Israel . Clubs are not evident in this field but the peripheral condensation of the internal filaments is well seen. Frequently several layers or rings of such condensed filaments are present arranged rather haphazardly and giving a bizarre untidy appearance. Though a ground substance is frequently faintly stained in sections of grains of this specie the filaments do not seem to be imbedded and part of this amorphous material as are the filaments of Nocardia brasiliensis. Plant's stain may reveal red borders to grains of S.madurae but no acid fastness can be found with modified Z.N.

Fig 56

of cases in the Sudan, the organism has never been observed there though it is not unknown in the neighbouring territories.

Isolates have been reported from the following areas: -

India (Carter 1874, Kanthack 1893, Ghosh et al. 1950, Andleigh 1954); Aden (Duncan et al. 1939); Somaliland (Vincent 1894); Ethiopia (Brumpt 1906); Cyprus (Williamson 1905); Israel (Altmann 1956); Greece (Kyriasides 1930, Sigalos 1943); Yugoslavia (Bordjoski & Milochevitch 1935); North Africa (Jouenne 1915, Catanei 1942); Belgian Congo (Courtois et al. 1945); South Africa (Welchman & Pirie 1921, Lurie 1955); U.S.A. (Green et al. 1948, Carroll 1949, Thompson & Vernon-Wax 1950); Cuba (Castenado 1955); Guinea (Williamson 1905); Mexico (Mackinnon 1955); Chile (Merino-Gonzalez 1946); Brazil (Yazbek 1920, Lacaz 1945, Mackinnon 1955); Argentina (Girardi & Khouryc 1946).

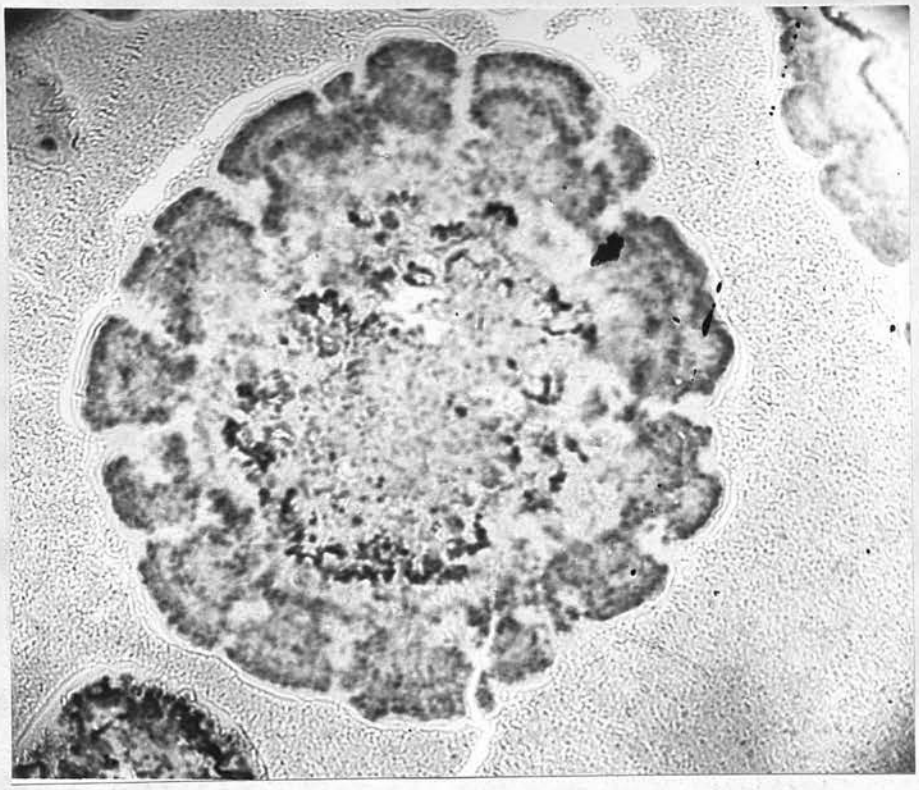
Doubtful cases have been reported from Hawaii, Straits Settlements and Dutch Guinea.

GRAINS.

I have been able to obtain sections from only two culturally proved cases, but I have seen many other sections which I consider to show grains that are characteristic examples. Amongst these are the grains studied by Kanthack.

The description of the grains that are to be found in the literature are very similar so that I consider that the specie is identifiable in histological preparations. This feature is in common with the other Streptomyces that cause mycetoma.

Grains are very variable in size and shape but not in basic structure or staining reactions. Grains may be denticulate, crescentic or show even more complex and bizarre formations. The latter is particularly the case in large granules which are of a greater size than those encountered in the other



Photomicrograph of a grain of a Tanganyikan mycetoma. The organism in this case is uncertain but the appearance is suggestive of Streptomyces madurae. The irregular denticulate border and the condensations of filaments in ring fashion is suggestive but some other features are not entirely typical.

Fig 57

Actinomycetes. The colour of the grains is usually stated to be white or pale yellow though a few authors have observed a faint pinkish hue. This may well be due to a surface smearing of sero-sanguineous fluid.

The centre of the grain is composed of loosely packed irregular filaments which are markedly Gram positive. Branching and bacilliform fragmentation of the filaments may be visible but there are no structures analogous to hyphae. In marked contrast to Nocardia brasiliensis and Streptomyces somaliensis the filaments are discrete and lie free from any interstitial cementing substance. Indeed some portions of a grain may be so loosely arranged that lacunar zones free from filaments may be seen. Surrounding the loose interior filaments is a condensed, densely staining, woven band of filaments. This enclosing structure, which has been likened by Kanthack to a mantle, stains very intensely with haematoxylin and is markedly Gram positive.

The periphery of the grain is moderately eosinophilic and is also red with Plant's stain. This area is somewhat amorphous, but with care a few clubs can be discerned in some part of a grain. Other regions of the rim are either entirely structureless or show ill defined elongated tape-like extension arising from the mantle. The latter are gram negative. No acid fastness is seen.

CULTURAL FEATURES.

The following description of cultural characteristics is based upon a comparitive study of eight isolates carried out by Mackinnon (1955).

Primary isolation is most readily obtained on an acid medium such as Czapek's (Duncan et al. 1939). The primary colony may show at first a red or pinkish hue but, this frequently disappears in secondary cultures as saprophytic existence becomes established (Vincent 1894, Duncan et al. 1939). As a rule

colonies are cream coloured and shiny. The latter feature which indicates absence of aerial mycelium is not invariable as is to be expected in a member of the genus Streptomyces.

On glucose peptone agar growth at 37° is luxuriant with the formation of creamy, crusted, and furrowed colonies. No pigment is produced in the medium even in the pinkish isolates. Microscopically a fine branching nonsegmented mycelium is observed. Inconstant swellings up to 1 u in diameter have been noted. Coccoid conidia are rare. Pleomorphic strains show a mycelium more fragmented and bacterial in form (Catanei 1942).

Colonies are proteolytic and hydrolyse starch. Glucose is utilised but not lactose. Other sugars investigated have given variable results but have shown no great activity. Urea, asparagine, histidine, and ammonium nitrate are capable of acting as nitrogen sources for the colonies.

Animal inoculation has rarely succeeded (Vincent 1894, Green et al. 1948, Mackinnon 1955) though Catanei (1948) found that pleomorphic strains showed virulence to guinea pigs.

SECTION XVII.

NOCARDIA BRASILIENSIS.

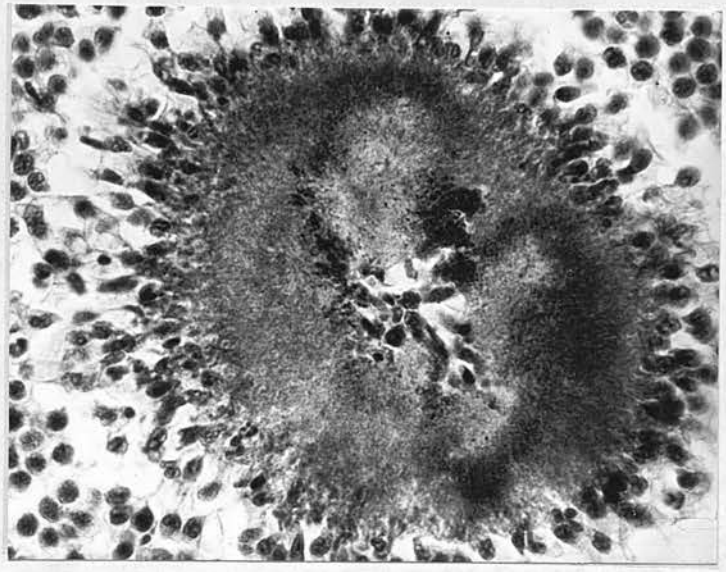
NOCARDIA BRASILIENSIS - LINDENBERG 1909.

SYNONYMS.

Discomyces brasiliensis	Lindenberg 1909
Streptothrix brasiliensis	Greco 1916
Actinomyces mexicanus	Boyd & Crutchfield 1921
Actinomyces brasiliensis	Gomes 1923
Oospora brasiliensis	Sartory 1923
Nocardia pretoriana	Pipjer & Pullinger 1927
Nocardia mexicanus	Oto 1928

Lindenberg described the organism as Discomyces brasiliensis and recorded the presence of acid fast filaments following growth in milk and the proteolytic activity that occurs in this medium. Yazbek (1920) after studying five new strains isolated in Sao Paulo and one received from Lindenberg, noted proteolytic activity on gelatin.

Castellani and Chalmers (1919), amongst other European mycologists who only knew the specie from descriptions in society communications, considered that Nocardia brasiliensis was identical with Nocardia asteroides. Lacaz (1941 & 1945) confirmed the original view that the two species were separate. In his numerous comparitive study of strains of the two organisms he found that the biochemical reactions of Nocardia brasiliensis were apparently very variable. Gonzalez-Ochoa (1945) carried out similar comparitive studies between isolates of Nocardia brasiliensis, Nocardia asteroides, and Nocardia mexicanus. As a result of this work he was able to show that Nocardia brasiliensis and Nocardia mexicanus were synonymous. These conclusions were based largely upon biochemical affinities. This had been shown by Lacaz to be perhaps unreliable. Nevertheless it seems to be generally accepted in the New World that the two strains are in fact but a single entity. Mackinnon (1955), in contrast to Lacaz, found a degree of constancy in biochemical reactions in the eighteen strains he observed though variation was

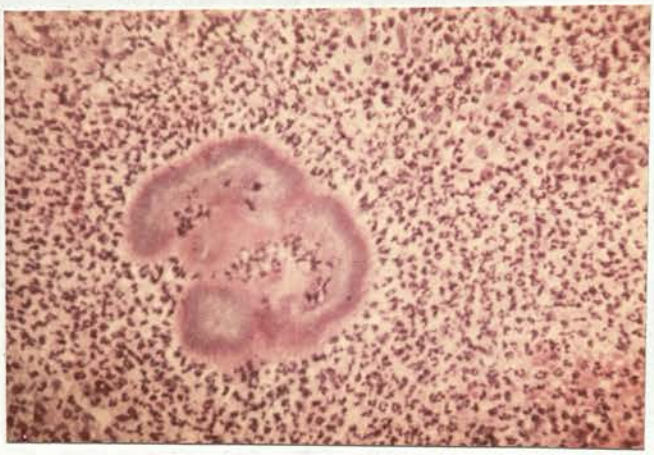


Photomicrograph of a grain of Nocardia brasiliensis. This section was cut from the block of the original case of Lindenberg. This particular grain shows few long filaments, most seem to have fragmented into coccal bodies. The threads seen in the inflammatory exudate do not form part of a grain. In this case no clubs are seen.

(Nature of stain uncertain.?Silver impregnation?)

x 580.

Fig 58



Grain of Nocardia brasiliensis stained with haematoxylin and oesin. The section was obtained from a case studied by Professor Flavio L. Nino of Buenos Aires, Argentina. Enlargement : x250 aprox.

Fig 59

presnet in the macroscopic and microscopic morphology. Proteolytic activity and the ability to ferment galactose was present in all the isolates. These latter findings are confirmed by the independent researches of Mariat & Levalle (1955).

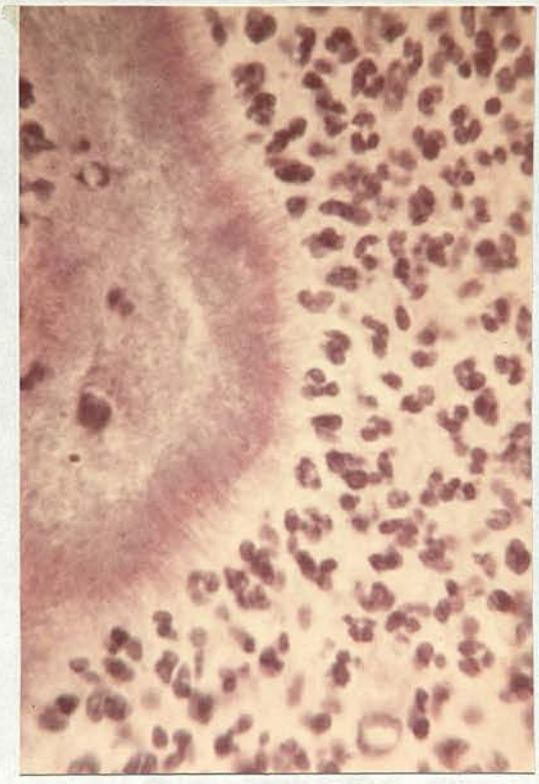
Moore et al. (1954) believe that much of the confusion that exists concerning this specie is attributable to the different meanings attached to the term acid-fastness. They state, that unlike Nocardia asteroides, acid fastness is not a feature of Nocardia brasiliensis unless it is grown on milk. To support his contention he quotes work carried out in the 1880's that showed that if an organism was grown on a lipid containing medium it would then appear to be acid fast. Though this may be so upon occasion, all strains studied by Mackinnon have shown semi-acid fastness when grown on conventional laboratory media.

GEOGRAPHICAL DISTRIBUTION.

The original isolation of the organism occurred in Brazil. Since that time the specie has been frequently recovered in Latin America. (Brazil : Lindenberg 1909, Yazbek 1920, Lacaz 1945); Venezuela: Montemayor (quoted by Mackinnon); Mexico: Gonzalez-Ochoa (1945-1951); Chile: Merino-Gonzalez. In the U.S.A. the organism has undoubtedly occurred on several occasions in the South Western States, but detailed mycological notes are lacking. Moore et al (1954) found the organism in a Texan and Bobbitt et al. (1955) in Boston.

Mackinnon (1955) considers that Nocardia pretoriana is this organism. This, if so, would establish the presence of Nocardia brasiliensis on the African continent.

Material I have obtained from East Africa and from Nigeria contains grains morphologically identical with Nocardia brasiliensis. Unfortunately in none of the five cases I have studied had any cultures been obtained.



Grain from a Tanganyikian mycetoma. The appearance is similar to that of Nocardia brasiliensis. No clubs are visible but within the eosinophilic periphery fine branching filaments are discernible. The photographic definition is poor but the illustration gives nevertheless a better impression of the microscopic staining characteristics than a verbal description. (H.& E.)

Fig 60

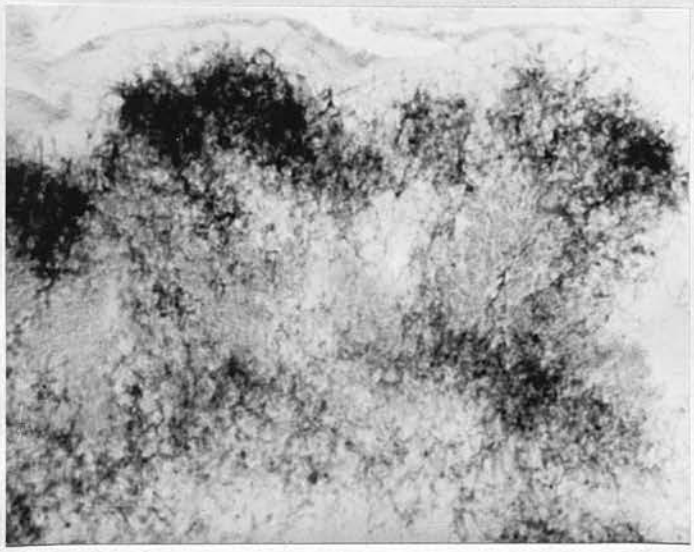
Acid fastness was marked in the tissue forms. Acquaintances of mine at Makere College, Tanganyika, and at Ibadan U.C.H. and Lagos, Nigeria, will endeavour to secure cultures from future cases. These are relatively infrequent, only three or four a year, but within a few years this point will be decided. On the basis of grain morphology I feel that the specie occurs in Africa.

GRAINS.

I have studied sections from only three proved cases. (Lindenberg's original, a case of Professor Nino of Buenos Aires, and a specimen sent by Professor Mackinnon). In addition I have reviewed the descriptions and photographs contained in various articles. Further to this are the five, as yet unclassified, cases from Africa.
The grains are variable in size but are on the average smaller than those of the Streptomyces. The grains are irregular in shape. Some are lobulated in a reniform manner but many others are less regular. Mackinnon has compared the appearance of these to such letters of the alphabet as C,S,U,L,N.

Clubs may occur but more frequently a peripheral oesinophilic substance surrounds the grain. This capsular material is acid or semi-acid fast. The nature of this peripheral band is obscure. It does seem to be part of the parasite and not merely a condensed mucinous exudate laid down by the host.

The centre of the grain is basophilic and granular. Filaments and small coccal bodies (?spores) are responsible for the stippled appearances as these structures are more basophilic than the cement substance. This latter property may form a basis for distinguishing grains of this specie from Streptomyces madurae. The grains of the latter also reveal central filamentous and coccoid bodies but these are more distinct and discrete as no detectable cement substance is visible.



Part of a Nocardia brasiliensis granule stained to show filaments. The ground cementing substance has not taken up the dye.
(Gram's stain. x650)

Fig 61

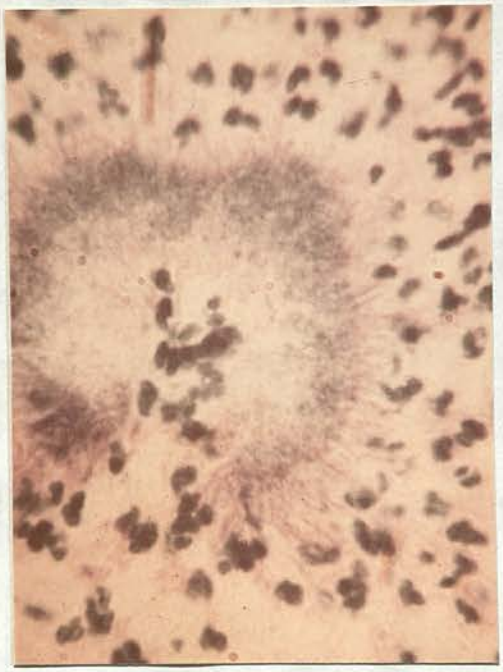
Grains resulting from experimental guinea pig infections are analogous to those found in human cases. Clubs occurred in testicular inoculations but not in peritoneal lesions. (Mackinnon).

CULTURAL FEATURES.

Growth is rapid over a wide range though 30°- 37° probably represents the optimum. On Krainsky's medium the colonies are heaped and furrowed. The edge of a colony is filamentous. The colour varies between sandy through orange to red. Pigmentation may also be noted in the medium.
Aerial mycelium is never very abundant. On liquid media a coarse membranous veil was formed.

Aerial mycelium does not differ from the vegetative mycelium. Filaments do not tend to fragment and stain well by Gram's method. Semi-acid fastness is a constant finding.

All strains show proteolytic activities but they do not hydrolyse starch. Asparagine glucose and galactose are metabolised but maltose sucrose and lactose are not. True fermentation is only observed with glucose and galactose.



Grain of Nocardia brasiliensis stained by a modified Ziehl-Neelsen technique. The filaments themselve are stained by the methylene blue counter stain.but,there is a peripheral zone of hyaline material which is acid fast.

Enlargement : 800 aprox.

Fig 62

SECTION XVIII.

NOCARDIA ASTEROIDES.

NOCARDIA ASTEROIDES - EPPINGER 1890

SYNONYMS.

Cladothrix asteroides	(Eppinger 1891)
Streptothrix eppingerii	(Rossi & Doria 1891)
Streptothrix asteroides	(Gasperini 1892)
Oospora asteroides	(Sauvageau & Radais 1892)
Actinomyces asteroides	(Blanchard 1894)
Discomyces asteroides	(Gedoelst 1902)
Streptothrix freeri	(Musgrave & Clegg 1907)
Discomyces freeri	(Yazbek 1920)

This organism rarely causes mycetoma though it is the most frequent cause of generalised nocardiosis. It was from a patient so afflicted that Eppinger demonstrated, in the brain, the presence of an aerobic, fragmenting, non sporulating actinomycete which was semi acid fast. At one time Nocardia brasiliensis was considered to be a synonym but the criteria by which it can be distinguished have been set out in the previous section. The organism reported by Musgrave & Clegg (1907) as Streptothrix freeri seems to have possessed the same characters as Nocardia asteroides and is generally regarded as a synonym.

GEOGRAPHICAL DISTRIBUTION.

This organism has been found in many parts of the world, in the large majority of instances from patients who have been suffering from a generalised Nocardiosis. Nocardia asteroides has been recovered from soil samples on several occasions. (Gordon & Hagan 1946).

It is noteworthy that out of the first 22 cases to be reported in the literature no fewer than 16 of the patients were in Europe. Since that date the large majority of isolates have been in the Americas. This suggests that cases are overlooked or are recorded as actinomycosis even though granules may not be evident. Several of the patients described by Cullen & Sharp (1951) were almost certainly infected by Nocardia asteroides. Panja (1955) has reported a case that may also have been due to Nocardia

asteroides. The last definite case reported from this country presented as a septicaemia simulating miliary tuberculosis (Hunter et al. 1954). Foulerton in 1910 published details of a case of Nocardiosis showing a similar clinical picture. These last two cases occurring in England indicate that, on rare occasions, systemic Nocardial infections may occur in Britain. The presence of more bacilliform and mycelial acid fast rods in the sputum than is usual in tuberculosis may be suggestive of the diagnosis before cultural investigations.

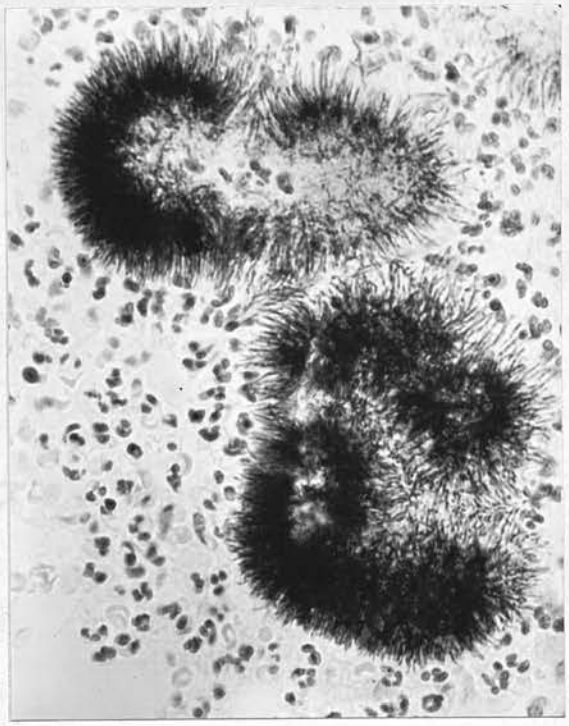
Nocardia asteroides has been isolated from patients with a localised mycetoma in India (Andleigh 1954); South Africa (Lurie 1955); Nigeria (Clark 1954); Panama (Calero 1947); Brazil and Uruguay (Mackinnon 1955); Israel (Altmann 1956); U.S.A. (Peters 1945, Bobbitt et al. 1955); and the Philippines (Musgrave & Clegg 1907). A case reported from Egypt by Massoud (1949) is also possibly due to this specie.

To the above I tentatively add a further case from Tanganyika. The diagnosis is based on the parasitic forms seen in sections.

GRAINS.

Grains never occur in generalised nocardiosis and are seldom seen in mycetoma due to this specie. Poorly organised loose tangles of short rods and branching filaments are common but true grain formation has, as far as I am aware, only been noted on four occasions in Nocardia asteroides infections. (Musgrave & Clegg, Peters, Calero, Mackinnon & Altmann loq.cit.). The tendency of this acid fast organism to fragment and disperse is in keeping with its taxonomic position which closely relates the Nocardia to the Mycobacteria.

I have not been able to study any section of grains from a proved case though I have read the descriptions in the literature and have some details of the appearances of the South American cases.



Grains from a Tanganyikan case of mycetoma stained by Gram. The filaments are strongly Gram positive and acid fast and have an appearance consistent with Nocardia asteroides. Bacilliform fragments and coccal bodies can be observed in the interior of the grains under the oil immersion lens.

Enlargement : 620. Case of Dr. J.P.Mackey, Senior Pathologist Dar es Salaam.

Fig 63

The morphology of the larger grains is uncertain but the smaller grains seem to show characteristics which are sufficiently constant and distinctive as to be highly suggestive of the specie.

The most striking feature is the presence of radiating Gram positive filament which merit the description star like. (Asteroides applies to the star appearance occasionally seen naked eye in giant colonies). The filaments themselves are semi acid-fast unlike those of Nocardia brasiliensis. Internally there is no basement membrane but merely ramifying small rods, bacilliform fragments, and coccal bodies. A grain may be hollow in the centre. Musgrave & Clegg noted a few peripheral clubs in large grains.

The photomicrograph which I reproduce is from an African. The filaments were strongly Gram positive and acid fast. I consider that the grains pertain to this specie as they conform in all particulars to the reported cases.

CULTURAL FEATURES.

The following brief notes are compounded from details available in standard works of bacteriology and papers by Hunter et al. (1954), Clark (1954) and Mackinnon (1955). The latter author has compared four strains, including a sub-culture of Eppinger's original isolate and two recent isolates recovered from South American cases of mycetoma.

The appearance of colonies frequently varies with age. The classical star like furrows are infrequently seen. Growth occurs aerobically on the usual media at an optimum temperature of 37°. Colonies are white to cream at first but gradually an orange, red or coral hue becomes evident but at no time is any pigment diffused into the medium. A powdery white surface is occasionally seen due to a non sporulating aerial mycelium.

Microscopically a fragmented mycelium can be recognised. A few branching elements may be noted

as well as short rod and coccal bodies. All these structures are Gram positive and, to a varying degree, acid fast. Carbol fuschsin is retained most tenaciously by the older parts of the culture - particularly the coccal bodies.

Proteolytic and amylolytic activity are absent. Sugars including galactose, are not fermented but glucose favours growth. The conventional sources of nitrogen are capable of being assimilated.

Most isolates have proved pathogenic to guinea pigs by either causing a septicaemia or more localised abscess systems which may spontaneously resolve. Musgrave & Clegg were able to reproduce mycetoma by subcutaneous injections of cultures into the feet of monkeys. Mackinnon's Uruguayan isolate produced grains with clubs following intra testicular inoculation of guinea pigs.

APPENDIX.

TABLE I.

Di Amino Diphenyl Sulphane.

	1	2	3	4	5	6	7
Phialaphora jeanselmei	o	+	o	o	o	+	++
Monosporium apiospermum	o	+	o	+	++	++	++
Cephalosporium falciformis	+	+	o	+	+	++	++
Madurella mycetomi 200	o	+	o	+	+	++	++
Madurella mycetomi 205	o	+	o	+	+	++	++
Madurella mycetomi 215	o	+	o	+	+	++	++
	$\frac{1}{500}$	P.A.B.A. $\frac{1}{500}$	$\frac{1}{5000}$	$\frac{1}{50000}$	$\frac{1}{500000}$	$\frac{1}{5000000}$	Control

Nutrient agar with serial dilutions of D.D.S. in acetone. pH - Approx. 6·8.

Column 7 represents control of nutrient agar + acetone + para amino benzoic acid. Column 2 shows results of D.D.S. + P.A.B.A.

TABLE II.

22' dihydroxy 55' dichloro diphenyl sulphide.

acetone diluent.

	A	B	C	D	E	F	
1	o	o	o	o	o	o	$\frac{1}{500}$
2	o	o	o	o	o	o	$\frac{1}{500}$ + PA.B.A.
3	++	++	++	++	++	++	Control
4	o	o	o	O	o	o	$\frac{1}{1650}$
5	o	o	o	o	+	o	$\frac{1}{5000}$
6	o	o	o	o	+	+	$\frac{1}{16500}$
7	o	o	o	o	+	o	$\frac{1}{50000}$
8	o	o	?	+	+	+	$\frac{1}{165000}$
9	o	o	o	+	+	+	$\frac{1}{500000}$
10	o	o	o	++	++	++	$\frac{1}{1000000}$
11	o	+	+	++	++	++	$\frac{1}{5000000}$
12	o	+	++	++	++	++	$\frac{1}{10000000}$

A = Phialaphora jeanselmei. B = Monosporium apiospermum
C.= Cephalosporium falciformis. D = Madurella mycetomi 200
E = Madurella mycetomi 205. F = Madurella mycetomi 215.

Nutrient agar pH 6·8.

MYCOSTATIN.

	A	B	C	D	E	F
Candida albicans	++	++	+	o	o	o
Torula histolytica	++	+	o	o	o	o
Monosporium apicspermum	++	++	++	++	+	o
Phialaphora jeanselmei	++	++	++	+	o	o
Madurella mycetomi	o	o	o	o	o	o
Madurella mycetomi	++	++	+	+	o	o

A. = Control.
B. = 3 units/ml.
C. = 10 units/ml.
D. = 30 units/ml.
E. = 100 units/ml.
F. = 300 units/ml.

++ = normal growth.
o = no growth.

Sabouraud glucose agar. Mycostatin dissolved in aqeous methanol mixture.

Readings taken after 3 days incubation at 37°C. The sensitivity of the two control organism is of the order normally reported.

INHIBITION OF MADUROMYCETES BY STILBAMIDINE.

4:4' - Diamidinostilbene diisethionate.

	A	B	C	D	E	F	G	H
Phialaphora jeanselmei	o	++	++	++	++	++	++	o
Monosporium apiospermum	o	++	++	++	++	++	++	+
Cephalosporium falciforme	++	++	++	++	++	++	++	+
Madurella mycetomi 200	+	++	++	++	++	++	++	+
MAdurella mycetomi 205	++	++	++	++	++	++	++	++
Madurella mycetomi 215	++	++	++	++	++	++	++	++

Concentration : -

A. = $\frac{1}{500}$ C. = $\frac{1}{5000}$ E. = $\frac{1}{50000}$ G. = Control

B. = $\frac{1}{1650}$ D. = $\frac{1}{16500}$ F. = $\frac{1}{165000}$ H. = $\frac{1}{500}$ +

P.A.B.A.

Dilutions made up with tap water. Growth on glucose agar slopes at pH of approx. 7·0 incubated at 37° for five days. If no growth was observed by the third day a further inoculation was carried out. In no case did this second seeding succeed.

INHIBITION OF MADUROMYCETES BY DIAMIDINODIPHENYLAMINE DIHYDROCHLORIDE. M. & B. 938.

	A	B	C	D	E	F	G	H
Phialaphora jeanselmei	o	o	o	++	++	++	+	++
Monosporium apiospermum	o	o	o	++	+	++	o	++
Cephalosporium falciformis	++	+	++	++	++	++	o	++
Madurella mycetomi 200	o	o	o	++	+	++	o	++
Madurella mycetomi 205	o	o	o	++	++	++	o	++
Madurella mycetomi 215	o	o	o	++	++	++	o	++

Concentration:-

A. = $\frac{1}{500}$ C. = $\frac{1}{5000}$ E. = $\frac{1}{50000}$ G. = $\frac{1}{500}$ P$^+$A.B.A.

B. = $\frac{1}{1650}$ D. = $\frac{1}{16500}$ F. = $\frac{1}{165000}$ H. = Control.

Dilutions made up with tap water. Growth on glucose agar slopes at pH 6·8 incubated at 37° for five days. If no growth was present by the third day further inoculation was carried out. In no case did this second seeding succeed.

INHIBITION OF MADUROMYCETES BY
2- HYDROXYSTILBAMIDINE ISETHIONATE.

	A	B	C	D	E
Phialaphora jeanselmei	o	o	+	++	++
Monosporium apiospermum	+	+	++	++	++
Cephalosporium falciformis	++	++	++	++	++
Madurella mycetomi 200	o	++	++	++	++
Madurella mycetomi 205	o	++	++	++	++
Madurella mycetomi 215	o	++	++	++	++
Concentration	$\frac{1}{500}$	$\frac{1}{1650}$	$\frac{1}{5000}$	$\frac{1}{16500}$	Control

Dilutions made up with tap water. Growth on glucose agar slopes at pH approx. 7·0, incubated at 37° for five days. If no growth was observed by the third day a further inoculation was carried out. In no case did this second seeding succeed.

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